MEDICAL MYCOLOGY

An Introduction to its Problems

BY

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PREFACE

Both medical men and mycologists incline to the view that medical mycology is a confused and somewhat esoteric subject. Medical mycologists are not of this opinion They would maintain that the apparent confusion must in large measure be attributed to the neglect into which this branch of medicine and mycology has fallen and the failure to appreciate the results of modern research. During the two past decades much has been done to dispel obscurities enveloping both clinical and mycological aspects of fungus infections in man and if, at the same time, unsuspected deficiencies in knowledge have been disclosed, stimulating prospects for future work have been revealed Much of the recent work is still scattered through many periodicals and no synthesis has yet been made This little book, which is neither a systematic account of pathogenic fungi nor an aid to the diagnosis of mycoses, does not adequately fill the gap It is, however, an attempt to provide students of mycology, medicine, and microbiology with an introduction to medical mycology by using the principal mycoses affecting man to illustrate some general problems of this borderline subject

I can claim only "amateur" status as a medical mycologist and this book owes much to the published work of others I am particularly grateful to Dr J T Duncan, formerly Reader in Medical Mycology in the University of London, for arousing my interest in fungi of medical importance and to all those professional medical mycologists who by conversation and correspondence have done so much to deepen this interest My very best thanks are given to the authors and publishers who have allowed me to reproduce their work, to friends who generously supplied me with photographs or material from which illustrations have been prepared, to my colleagues for their criticism of the manuscript, and to my wife for help with the chapter on poisonous fungi and for making drawings

G C A

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CHAPTER 1

INTRODUCTORY

The beginnings of medical mycology can be traced to Western Europe just over a century ago It was in 1835 that the Italian, Agostino Bassi (1773–1856), first offered experimental proof that one living organism could cause disease in another by his demonstration that the muscardine disease of silkworms was caused by the fungus now known as *Beauveria bassiana* Within the next ten years the fungi associated with favus and thrush in man were described Interest in fungi as pathogens of man was thus aroused but before the controversy was finally settled as to whether such fungi were the result rather than the cause of disease, the bacteriological discoveries of Pasteur, Koch, and Cohn brought about a change of emphasis The subsequent recognition of the undoubted importance of protozoa and viruses as pathogens of man completed the almost total eclipse of the consideration of fungi in etiology, an eclipse from which medical mycology is only now emerging

No medical mycologist would claim that fungi rank in importance with bacteria and viruses as a cause of disease in man Fungus diseases may, however, be of greater importance than hitherto supposed, for the effects of those already known are not unimpressive Emmons (1948) recently drew attention to the fact that of the 1,385,187 deaths recorded in *Vital Statistics of the United States for 1942*, 359 were attributed to fungi Although this is less than 0 03 per cent of the total, it is nearly twice as many as all the deaths during the same period from paratyphoid fever, undulant fever, smallpox, rabies, leprosy, plague, cholera, yellow fever, and relapsing fever It is greater than that from all the typhus-like diseases and more than half the number from typhoid fever, tetanus, or poliomyelitis The 1945 returns show a similar pattern (Salvin, 1950a) It should be noted, however, as Emmons points out, that effective prophylactic and control measures prevent deaths from many better known diseases while no control measures are known for several fungus diseases which are usually fatal On the other hand, lack of knowledge might involve attributing some deaths due to fungi to other causes and a number of the most widespread fungus diseases are not fatal

Reliable statistics are not available for other countries. A preliminary survey made during the war showed fungus diseases to be of common occurrence in Britain (Duncan, 1945) where, according to a recent compilation, more than sixty species of fungi have been claimed as pathogenic for man and animals (Ainsworth, 1950, 1951)

It was in the United States at the turn of the century that the first mycological plant pathologists were appointed by a Department of Agriculture and it was there about twenty years ago that the first trained mycologist was appointed by a Public Health Service. Most countries have followed the example of the United States in the field of plant pathology Very few have yet appointed their first medical mycologist. This state of affairs is to be deplored because the significance to public health of the recognized fungus diseases is often still obscure and the possible implication of fungi merits consideration in any disease of uncertain cause

Disease Nomenclature

Fungus diseases of man and animals, unlike the more numerous bacterial diseases of man and fungus diseases of plants, are distinguished by a special name In all languages, such a fungus infection is known as a *mycosis* Mycoses are commonly differentiated by the use of a prefix which is either derived from the part of the body affected or from the name, usually the generic name, of the pathogen involved For example, a mycosis of the skin is termed a *dermatomycosis*, one of the bronchi a *bronchomycosis*, and one of the nails an *onychomycosis*, while the diseases resulting from an actinomycete and from *Coccidioides immitis* are designated *actinomycosis* and *coccidioidomycosis*, respectively An alternative method for coining disease names is by adding the suffix -*osis* to the generic name of a pathogen as in *aspergillosis* (from *Aspergillus*), *microsporosis* (*Microsporum*), etc. Others are distinguished on a geographical basis, e g North American blastomycosis which must not be confused with South American blastomycosis or with European blastomycosis (torulosis)

However derived, a number of disease names have an international currency which is liable to mislead an unwary clinician into the belief that the meanings also have an international uniformity Unfortunately, this is not always so Many disease names are applied to conditions induced by more than one pathogen and if used without any indication of the particular fungus involved, are a fruitful source of misunderstanding

Pathogenic Fungi

The fungi which cause mycoses are many and diverse. To describe these fungi in detail is outside the scope of this review. So too, is a detailed account of fungi in general for which the reader is referred to any of the standard mycological textbooks (e g Bessey, 1950) To appreciate the significance of what follows it is necessary only to [illegible] of vegetative filaments is known as *mycelium* and the individual filaments as *hyphae* The characteristic unit of reproduction is the *spore* and the four main Classes of Fungi are distinguished by the method of spore formation In three Classes spore formation may be preceded by nuclear fusion In the Basidiomycetes this sexual fusion takes place in a special unicellular structure, the *basidium*, on which sexual spores (*basidiospores*), usually four in number, are borne exogenously (*see* Fig 7, p 91), whilst in the Ascomycetes, typically eight *ascospores* are formed endogenously in a somewhat similar organ known as the *ascus* (Plate I (7–21) and Fig 2) Sexual reproduction in the third and most primitive Class, the Phycomycetes, results in thick-walled resting spores In addition to sexual, or perfect spores, members of these Classes may give rise to asexual or imperfect spores Sometimes there is a regular alternation of perfect and imperfect states, sometimes one spore form predominates Many fungi are only known to produce [illegible] unrecognized, some are the imperfect states of known perfect fungi and are classified in the Fungi Imperfecti for the sake of convenience, and others, the great majority, are fungi which are believed to be unable to reproduce sexually

In addition to the fungi proper, mycologists, and particularly medical mycologists, frequently deal with certain actinomycetes, organisms which show characters in common with bacteria, such as the tubercle bacillus, on the one hand and with Fungi Imperfecti on [illegible] anaerobic *Actinomyces* and the aerobic *Nocardia*, break up into short rod- or coccus-like elements which function as spores (*arthrospores*)

Fungi are characteristically either parasites of plants or saprophytes of plant products Plant-parasitic fungi are well represented in all Classes of the Fungi and several important Orders and Families are composed exclusively of such forms Some major groups such as the Rusts (*Uredinales*) are obligate parasites of plants, other Orders of plant pathogens, such as the Smuts (*Ustilaginales*), can be grown in artificial culture, when the growth may be very different from the mycelial parasitic phase, whilst for many fungi the transition from the parasitic to the saprophytic mode of life, or *vice versa*, can be very easily effected

Fungi pathogenic for man and animals show an interesting contrast They are unrepresented in the Basidiomycetes, a Class which does, however, include fungi poisonous for man The pathogenic status of the few undoubted Phycomycetes found associated with diseased conditions is often very doubtful, as for example, is that of *Absidia corymbifera*, a *Mucor*-like mould sometimes isolated from the ear of man Ascomycetes, also, are rarely pathogenic, and the few that are do not usually produce ascospores in the parasitic state An interesting exception is *Piedraia hortai* which causes a disease of the hair in the tropics known as Black Piedra. This weakly pathogenic fungus develops as small black unsightly nodules on the hair shaft and causes no serious discomfort but it is remarkable in producing asci and ascospores within the concretions while still attached to the living hair (Plate I) It is possibly related to the Asterineae, a group of tropical ascomycetes which grow parasitically in rather a similar way on the surface of leaves. Most of the important fungus pathogens of man are imperfect forms and although some can be classified without difficulty, the relationships of others are very obscure

Another contrast with plant-pathogenic fungi is the rarity, or perhaps absence, of obligate parasitism A few actinomycetes and *Rhinosporidium seeberi*, the cause of polyp-like growths in the nose and other organs, have not been cultured but most of the other known fungi pathogenic for man and animals grow well on artificial media *in vitro*

It was Nickerson (1947) who first drew attention to the striking contrast between the nutritional requirements of pathogenic fungi and pathogenic bacteria. Among bacteria increasing pathogenicity is correlated with decreasing synthetic ability and in a genus containing both saprophytic and pathogenic forms the latter invariably have the more exacting nutritional requirements Plant pathogenic fungi which

are obligate parasites also exhibit marked nutritional specialization. Fungi pathogenic for man, on the other hand, show a negative correlation between pathogenicity and specialized nutrition This is illustrated in Table I where the nitrogen and growth factor requirements of several pathogenic fungi are summarized *Pityrosporum ovale*, the 'bottle bacillus" of Unna is clearly the most specialized of the series for in addition to the need for organic nitrogen it requires thiamin (vitamin B1) and is the only known micro-organism for the normal growth of which a trace of fatty acid is essential. This minute yeast is usually found associated with dandruff scales It has been claimed to be a contributory cause of seborrhoeic dermatitis and other defects of the skin but its role in these disorders is very obscure and its usual *status* is probably that of a highly specialized saprophyte At the other extreme is *Blastomyces dermatitidis*, a virulent pathogen, which can make satisfactory growth in a simple glucose-mineral salts medium. The ringworm fungi and the mycelial yeasts show nutritional and pathogenic characteristics which are intermediate between these extremes

Geographical Distribution

A few mycoses have a world-wide distribution Actinomycosis (*Actinomyces israeli*) has been diagnosed "wherever there is a microscope and a laboratory" (Cope, 1938, p 27) by the Gram positive, acid fast granules composed of delicate branching hyphae, often with characteristic peripheral "clubs," found in pus from tissues and in tissue sections (Plate VI (3)) (In passing it may be noted that the failure to supplement microscopical observations with critical cultural studies has resulted in uncertainty regarding the species of *Actinomyces* involved A single species was thought to cause actinomycosis of man and cattle According to the recent work of Erikson (1940) and Thompson (1950), there are almost certainly two) Moniliasis (caused by species of *Candida*) in its various manifestations is also ubiquitous Both these diseases are what is termed *endogenous* That is, the causal organisms may be isolated from apparently normal individuals and are found very rarely, *if* ever, unassociated with man or animals. In contrast to this are the *exogenous* diseases in which infection is incurred from an outside source For some diseases, such as sporotrichosis (*Sporotrichum schenckii*), the pathogen is a recognized saprophyte, for others, such as South American blastomycosis (*Paracoccidioides brasiliensis*) which is believed to be of exogenous origin, no external source of infection has

mycoses The differential characters of these two groups as summarized by Henrici are set out in Table II

TABLE II

DIFFERENTIAL CHARACTERS OF SUPERFICIAL AND DEEP-SEATED FUNGUS DISEASES

	Superficial Mycoses (dermatomycoses moniliasis)	Deep-seated Mycoses (blastomycosis torulosis, etc.)
Normal habit of growth of the pathogen	Parasitic	Saprophytic
Lesions	Mild, superficial, restricted	Severe deep spreading
Age incidence	*Usually young children* and young adults	Usually *older people*
Occupational *incidence*	*No* particular occupations	*Occupations* exposing the tissues to soil or vegetable matter by wounds or inhalation
Geographical distribution	World-wide not restricted	Restricted or if world-wide much more prevalent in certain areas
Course	Self limited, rarely fatal	Progressive often fatal

(*From* Henrici (1940) Table I p 115)

Two points may be emphasized The first is that the fungi *responsible* for superficial mycoses are normally parasites of man and animals and are well adapted for this mode of life The diseases they cause are typically endogenous Many of the fungi responsible for the systemic mycoses, on the other hand, are believed to live normally as saprophytes and in general they are less well adapted for parasitism The second, and perhaps more interesting point, is that the course of superficial mycoses follows a similar pattern to that of most bacterial and virus diseases. The incubation period is relatively short, the onset of the disease sudden, and the symptoms, which are severe at first decrease in severity with time and so spontaneous healing results Deep-seated mycoses, in contrast, show similarities to aberrant bacterial diseases such as tuberculosis and leprosy caused by species of *Mycobacterium* The incubation period is protracted, the onset of symptoms insidious, and the subsequent course one of increasing severity which not infrequently terminates in death.

Both types of fungus disease, however, show a common feature in that they induce an allergic state in the infected subject (*see* Chapter VI) According to Von Pirquet, a parasite is not injurious directly but only after the cells of the host have become sensitized to its products Alternatively, according to Pfeiffer, the invading organism contains within its protoplasm endotoxins which act on the host tissues after death and disintegration of the cells of the parasite These views are not, as Henrici points out, mutually exclusive *Aspergillus fumigatus* which immediately produces acute disease when inoculated into experimental animals contains an endotoxin, and so too, probably, does *Candida albicans* which produces a similar result. Endotoxins also occur in non-pathogenic fungi such as *Rhizopus stolonifer* but the occurrence of these toxins is not usual among pathogenic fungi, massive doses of which cause no toxic reaction Experimental evidence obtained by Henrici with *A fumigatus* suggests that sensitization may play a part in infection by this fungus for there are two modal times for death of experimental animals, at one to two days with large doses, and with smaller doses at about the tenth day, when the lesions change from abscesses to tubercles (*see* p 46) It may be that it is about the tenth day that an infected animal becomes hypersensitized to the products of the fungus It is at this time that the skin tests become positive

In dermatomycoses, after a lesion has developed sufficiently the skin becomes hypersensitive so that an almost identical lesion can be produced by inoculation with nitrogen-containing polysaccharide extracted from the fungus On the other hand, a hypersensitive animal may be immune to re-inoculation or the new lesion develops much more rapidly

Henrici suggests that these apparently contradictory effects of the allergic state the injurious and the protective may be due to the seat of the allergic reaction Inoculation with tuberculin will protect a guinea-pig against re-inoculation with the tubercle bacillus but a large injection of tuberculin may lead to an extension of pulmonary tuberculosis in man In one case the organism and products of the reaction are cast off to the exterior in the other they are liberated into the tissues In this manner an allergic reaction in the skin may be protective, in the lung or kidney it may result in extension of the disease Only pathogenic fungi appear able to induce hypersensitivity This type of sensitivity is not the usual anaphylactic sensitivity which prevails in diseases such as asthma and hay-fever Like sensitivity to tuberculin, it is not accompanied by the development of humeral antibodies

to replace Tokelau itch, because the infected skin scales appeared to be imbricated, i e like overlapping tiles It was nearly twenty years later that the French worker Blanchard named the fungus *Trichophyton concentricum* after the concentric patterns which it caused on infected skin (Plate III)

The recognition of Tokelau itch as a mycosis was the result of discoveries made in Europe some thirty-five years before by a number of workers but particularly by David Gruby [1810–98] who may justly be claimed as the founder of medical mycology Gruby was a Hungarian Jew who studied medicine in Vienna and in 1840, rather than submit the certificate of baptism necessary for a professorial chair,

was not, however, fully appreciated until the last decade of the century when Raymond Sabouraud [1864–1938] began his classical studies on the ringworm fungi by pure culture methods, studies which by their magnitude and authority gave Sabouraud an unique position in twentieth century medical mycology

that is to say res of a is no reaction until the skin is scratched when a mycotic lesion develops at the point of injury The action of some dermatophytes is confined to the skin, as for example, is that of *Trichophyton concentricum* *Epidermophyton floccosum* (a cause of ringworm of the groin and feet) is also unable to attack hair, although it occasionally invades the nails, but most species are able to parasitize both skin and hair

The current nomenclature of the ringworm diseases has a topical basis Ringworm of the head is designated *tinea capitis*, ringworm of the feet as *tinea pedis*, that of the body as *tinea corporis* and so on Although these names are valuable to the clinician they lack precision because more than one fungus is able to cause disease at any one site It is not always clear whether similar clinical conditions caused by different pathogens should be given the same therapeutic treatment There is general agreement, for example, that tinea capitis in children due to *Microsporum audouini*, a fungus found naturally only on man, is more difficult to cure than the very similar condition caused by *M canis* from cats and dogs These two types of microsporosis are distinguished with certainty only by culturing the pathogen, but sometimes

an experienced clinician is able to distinguish the latter by its more inflammatory reaction which may on occasion be so severe as to induce the type of lesion known as a *kerion* Body ringworm in man resulting from the cattle ringworm fungi may also be pustular and such a reaction is a general characteristic of animal ringworm fungi in man. Dermatophytes found naturally only in man tend to cause less inflammation than those from animals

Epidemiology

A detailed consideration of the epidemiology of ringworm would here be out of place It is only necessary to illustrate the importance [illegible]

another In France, species of *Trichophyton* predominate, in eastern Europe, Asia Minor, and North Africa other species of the same genus replace those commonly found in western Europe, whilst in the British Isles the fungi most frequently involved are two species of *Microsporum*, *M audouini* and *M canis* (*see* Walker, 1950)

Head ringworm, being popularly associated with uncleanliness, is still considered by middle and upper class mothers to be a somewhat disreputable disease The animal type of *Microsporum* may, however, be introduced into families in all strata of society by infected pets Large outbreaks caused by this species sometimes originate by many children having access to one source of infection such as the school cat, although most infections are of one child by another *M canis* is generally amenable to topical treatment, or is eliminated spontaneously as a result of the inflammatory reaction induced, and thus shows a marked contrast to the very intractable *M audouini* which is responsible for the major epidemics among children of school age

M audouini, which was first described by Gruby in 1843, appears to have originated in western Europe Its incidence falls off in eastern Europe and the only records from tropical countries are infections in children of European residents It has, presumably as a consequence of emigration, become firmly established in the temperate parts of North America and in Australasia where, as in Britain, epidemics are not infrequent.

The signs of the disease are hairs broken off a few millimetres above the scalp to leave stumps surrounded by a loose sheath of spores by

baths) are high and the hot and humid condition of the feet during working hours favours the growth of the fungus

The Ringworm Fungi

Ringworm fungi when parasitizing skin rarely show any diagnostic characters Possibly owing to the low nutritive value of keratinized tissue, the growth in skin is chiefly mycelial and the septate, branched hyphae of one species as seen in a scale cleared for microscopic examination by *mounting in a 20 per cent solution of caustic potash (KOH)* are very similar to those of another (Plate III (2)) The morphology and arrangement of the spores (arthrospores) resulting from the fragmentation of hyphae are also non-specific in skin although the size and disposition of arthrospores developed on an infected hair may give valuable clues to the identity of the pathogen (Fig 1, h–j, Plate V (1)) It is only by growth in pure culture on artificial media that the full potentialities of a dermatophyte are revealed and no dermatophyte can be identified with certainty until its cultural characters, both macroscopic and microscopic, have been determined

The nutritive requirements of the ringworm fungi are not unduly exacting (*see* p 6) Provided nitrogen is available in an organic form, such as that offered by the amino-acid mixture of a peptone, most dermatophytes will make profuse growth on a great variety of media *and in order that the results obtained by one worker might be* correlated with those of another many attempts have been made to standardize diagnostic culture media The most widely used medium is still the peptone-sugar medium known as "Sabouraud's Agar" which was developed by Sabouraud in the eighteen nineties Unfortunately, Sabouraud used a particular French peptone, Granulée de Chassaing, which has not been generally available since the First World War, in combination with a special brand of impure maltose (Brute de Chanut) or glucose (Massée de Chanut) Later workers have shown that pure glucose or maltose may be substituted for that recommended by Sabouraud and that good results are obtained with other types of peptone, but a really satisfactory standard medium which could be prepared in any mycological laboratory has yet to be devised

The importance of the composition of the medium is in part due to the emphasis that Sabouraud, and others who undertook the first pure culture-studies of the ringworm fungi, placed on macroscopic cultural characters such as whether the colony is cottony (floccose, lanose),

powdery, or smooth (glabrous) and the pattern of the folds or con- [illegible] *Le Teignes* 1910 (*see* Plate II) Since the publication of that mycological classic, medical men with little knowledge of mycology have tended to make their identifications by matching cultures against Sabouraud's figures without any attempt to ascertain the characters of the spores and other microscopical features which are, as Sabouraud knew, of great taxonomic importance

Dermatophytes produce three types of asexual spores in culture [illegible] conditions not yet fully understood They are often most readily produced in a primary culture (the culture derived from an inoculum of infected material) and sometimes, as in *Microsporum audouini* and *Trichophyton violaceum*, it is the lack of the appropriate growth substances which prevents their development (Hazen 1947 Georg 1948) Among other microscopic structures mention may be made of hyphae composed of cells each dilated at one end, the so-called 'racquette hyphae,' spiral hyphae (*see* Fig 1) and nodular organs' which are possibly vestigial ascogonia

The dermatophytes are characteristically unstable in culture After a shorter or longer interval the culture becomes overgrown by a cotton wool like mycelium which finally replaces the normal growth (Plate II) This phenomenon is known as *pleomorphism* and pleomorphic cultures develop no macroconidia and few if any microconidia or other diagnostic structures Pleomorphic cultures are still patho- [illegible]

Empirical work on the development of media for the prevention of pleomorphic change, has shown that sugar-containing media encourage pleomorphism which is at least delayed by culture on natural media, such as wheat or barley grains, containing complex carbohydrates and on Sabouraud's Conservation Agar, a 3 per cent peptone medium containing no sugar

characteristic of clinical favus *Microsporum* was differentiated by the mosaic-like sheath of small spores around the hair shaft from *Trichophyton* in which the spores were arranged in linear series The genus *Trichophyton* was sub-divided into two series according to whether the fungus developed on the surface of the hair (Ectothrix) or within the hair (Endothrix) (Fig 1, *i*, *j*) Twenty years later Sabouraud revised this classification and, while still maintaining the importance of clinical criteria, replaced *Trichophyton* by *Endothrix*, *Microides*, and *Megaspore* (Sabouraud, 1929), three genera which have been little used, for most of Sabouraud's followers prefer his earlier groupings and names

Grigoraki may be cited as an author who took up an extreme mycological standpoint and devised an elaborate classification, based on the macroscopic and microscopic cultural characters and necessitating the creation of six new genera (Grigoraki, 1925, Guiart and Grigoraki, 1928) Vuillemin (1931) distributed a number of the ringworm fungi among "non-medical" genera of the Fungi Imperfecti and C W Dodge (1935) employed eight genera and numerous sub-genera in the primarily mycological classification which he devised

There is a general feeling among mycologists that genera of fungi are best delimited on morphological grounds and the most useful modern advance in the classification of the dermatophytes was the demonstration by Emmons, a mycologist by training, that three of Sabouraud's genera could be satisfactorily defined in mycological terms (Emmons, 1934) In Emmons' scheme the most important diagnostic criterion is the character of the macroconidium In the genus *Microsporum* the macroconidium is more or less spindle-shaped multiseptate, and thick-walled, club-shaped, multiseptate, and thin-walled in the genus *Trichophyton*, and pear-shaped, with no, or only a few, septa in *Epidermophyton*, while the absence of microconidia in *Epidermophyton* and other characters which served to differentiate the three genera were shown to be correlated with macroconidial characters (*see* Fig 1) The species which Sabouraud included in the genus *Achorion* were found to be naturally accommodated in *Microsporum* and *Trichophyton* as delimited by Emmons and *Epidermophyton* was reduced to a monotypic genus for *E floccosum* (syn *E inguinale*)

The generic proposals of Emmons have been widely accepted in North America and elsewhere Medical mycologists of the French school, however, frequently follow the classification employed by the late Professor Langeron, two of whose generic uses call for comment. Langeron and Milochevitch (1930) interpreted the conidial masses

associated with branched antler-like hyphae exhibited by certain species of *Trichophyton* as perithecia and considered that it would make for a more "natural" classification if these species were classified, not as Fungi Imperfecti but as Ascomycetes, that is to say, as Perfect Fungi The relationship of the dermatophytes to ascomycetes such as those of the family Gymnoascaceae had long been considered a not unreasonable speculation and it was to the genus *Ctenomyces* of that family that Langeron and Milochevitch transferred these species. Langeron's other innovation was to widen the genus *Microsporum*, as a new genus *Sabouraudites*, so as to include certain species of *Trichophyton* (Ota and Langeron, 1923)

Dermatophytes have been classified in more than thirty genera in addition to those already mentioned Most of these genera were specially proposed for ringworm fungi, but most of them have never been generally used, even by their authors The relationship of the half dozen generic names used by Sabouraud, Emmons, and Langeron are summarized in Table III

Sabouraud distinguished species mainly by the character of the growth in culture and by microscopical characters, and most modern mycologists would accept the validity of these criteria even though few would agree that all the species proposed by Sabouraud can be maintained This change of viewpoint is in large measure due to the many investigations on the range of variation exhibited by fungi and by micro-organisms in general The studies by Emmons on spontaneous variation in *Microsporum gypseum* and *Trichophyton mentagrophytes* indicated the very wide differences between variants produced from [illegible]

differences in respect of vitamin requirements between strains within the species *T discoides* and *T ochraceum*

Emmons and Hollaender concluded that "species lines have been drawn too narrowly among dermatophytes and that some so-called [illegible]

limit. If the "splitting" of species as advocated by Sabouraud makes precise identification of the fungus difficult if not impossible, extreme

paper on ringworm and finds that the only clues to its contents may be the illustrations and the specific names of the fungi in the familiar Latin Hence the need that the names selected should be internationally acceptable Unfortunately, medical mycologists have shown a regrettable ignorance of the rules of nomenclature, or they have wilfully infringed the rules in a misguided effort to avoid changes in names which, though familiar, frequently lacked precision or were merely parochial

The two main objects of the International Rules are to ensure that each fungus has but one name in any group in which it may be classified and to ensure precision in the application of names

The first objective is attained by the familiar "law of priority" The cat and dog ringworm fungus, to take a well-known example, was named *Microsporum canis* by Bodin in France, in 1902, *M felineum* by Mewborn in New York, also in 1902, and *M lanosum* by Sabouraud in 1907 Mewborn refers to Bodin's book and so Bodin's name takes priority *M canis is, therefore, the valid name for this fungus when* classified in the genus *Microsporum* Cats may be more frequently infected than dogs but the rules do not take into account the appropriateness of names *M felineum* could only be correctly used by an author who believed that different species infect cats and dogs, respectively *Similarly, there is general agreement that Sabouraud in 1910* gave the name *Epidermophyton inguinale* to a fungus described many years earlier by Harz as *Acrothecium floccosum* so that the valid name for the fungus studied by Sabouraud, if Sabouraud's taxonomy is accepted is *E floccosum* (Harz) Langer and Milochev, in spite of the fact that Langeron and Milochevitch did *not transfer Harz's species to Epidermophyton* until 1930.

The selection of a valid name involves the application of a set of arbitrary rules Most ringworm fungi have more than one valid name and the choice between these involves personal judgment *Achorion schoenleini* and *Trichophyton schoenleini are both valid names for the* favus fungus The first is used by workers who accept a genus based on clinical characters, the second by those who think such practice undesirable Again, *Trichophyton mentagrophytes* and *Ctenomyces mentagrophytes* are both valid and the second name is used by those in whose judgment the absence of ascospores does not prevent the classification of this species as a perfect fungus.

Precision in the use of names is ensured by the type method Every taxonomic group is based on a type, a family on a type genus, a genus

on a type species, and a species on a type specimen which may be an actual specimen or may be a published description, often with illustrations The application of a name must always include the original sense as indicated by the type It may include much more as, for example, when *T schoenleini* is used to cover all the faviform species of *Trichophyton* Such usage is valid until the scope includes an older taxon, the name of which then takes priority Ota and Langeron's generic name *Sabouraudites* is invalid for this reason. It includes the older genus *Microsporum* of which it is an obligate synonym since *Microsporum* and *Sabouraudites* are based on the same type species The [illegible] a good reason for its rejection

A stable taxonomy is a prerequisite for a stable nomenclature and for the dermatophytes as for many other groups of fungi a generally agreed classification is still far off An attempt to freeze nomenclature on the basis of any current taxonomic system is clearly premature and such action would be intolerable to systematists who must retain their right to devise new and perhaps more natural classifications as knowledge of fungi and their inter-relationships advances and thus to change names Workers in branches of applied mycology such as plant pathology and medical mycology, make frequent use of relatively few names and it is changes in these names which raise tempers and when very frequent, make for confusion The latest views of even the most eminent systematist are not necessarily final and it is often advisable to treat them with a certain scepticism Modifications in classification involving name changes do not invalidate earlier names which do not infringe the Rules of Nomenclature and, as already pointed out, a species may have several internationally valid names. It may therefore, assist uniformity of usage if acceptance of a proposal to change the name of an important pathogen is deferred until it is known whether the new classification is acceptable to other systematists interested in the particular group of fungi involved Even if there is agreement among systematists, a sound knowledge of the Rules of Nomenclature and action by an International Botanical Congress may render changes in the names of important fungi unnecessary For the past fifteen years [illegible]

can the presence of mycelium be demonstrated but the arrangement of the whorls of budding cells (blastospores) can also be conveniently studied An alternative method, which depends on the culture medium preserving the mycelial structure of the submerged growth is to take a four- to seven-day-old slope culture, wash off the surface growth remove the whole slope from the tube, and then mount thin transverse sections in lactophenol (cf Fig 2, *f*, *g*, *h*)

It is frequently difficult to induce ascospore formation in a sporogenous yeast and a number of different techniques is available Almeida and Da Silva Lacaz (1940) advocate incubation for 24-48 hours in sterile 5 per cent potato starch solution for this purpose and their technique has the additional advantage of revealing mycelial growth at the same time

Morphology is of limited value in distinguishing species of yeasts In the genus *Candida* the production of thick-walled spherical chlamydospores in corn (maize) meal agar differentiates *C albicans* (although the absence of these structures does not exclude this species) and the flat or wrinkled, dry growth of *C krusei* on Sabouraud's or on beer wort agar is characteristic Usually, species are more certainly differentiated by carbohydrate fermentation tests similar to those employed for differentiating bacterial species but with the various sugars at a concentration of 2 per cent The four sugars—glucose, sucrose, maltose, and lactose—are now generally considered sufficient for the recognition of the commoner pathogenic and non-pathogenic species of *Candida* Castellani (1937) by using thirteen carbohydrates and other special tests, recognized more than thirty species but most of these are no longer accepted The diagnostic characters of three common species of *Candida* are summarized in Table IV

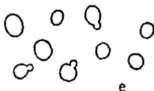
Yeasts of Medical Importance and their Nomenclature

With a few exceptions, the yeasts of medical importance are fungi imperfecti The cause of torulosis (see p 48) is an asporogenous, non-mycelial yeast sometimes classified in the genus *Torulopsis* but which, if the arguments recently advanced by Skinner (1950) are accepted, is best designated *Cryptococcus neoformans* *Pityrosporum ovale* (see p 5), belongs to the same group The most common and ubiquitous pathogenic yeasts, however, are characterized by mycelium (or more accurately, pseudomycelium, for the hyphae may be interpreted as elongated budding cells) and belong to the genus *Candida*

YEASTS

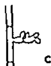
ASCOMYCETES	FUNGI IMPERFECTI
Order: Endomycetales	Order: Moniliales
Family Saccharomycetaceae	Family: Pseudosaccharomycetaceae
(Sporogenous Yeasts)	(Asporogenous Yeasts)

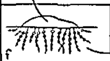


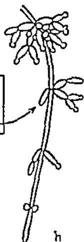












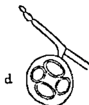




Fig. 2. Sporogenous and Asporogenous Yeasts

Saccharomyces a vegetative cells b, four ascospores in an ascus.
Endomycopsis c, hypha with budding cells, d ascus (after Guilliermond)

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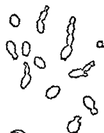
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Yeasts of Medical Importance and their Nomenclature

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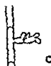
YEASTS

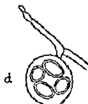
ASCOMYCETES	FUNGI IMPERFECTI
Order Endomycetales	Order Moniliales
Family Saccharomycetaceae	Family Pseudosaccharomycetaceae
(Sporogenous Yeasts)	(Asporogenous Yeasts)



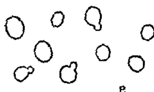


Saccharomyces





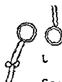
Endomycopsis



Cryptococcus









Candida

FIG 2. SPOROGENOUS AND ASPOROGENOUS YEASTS

Saccharomyces *a* vegetative cells *b* four ascospores in an ascus
Endomycopsis *c* hypha with budding cells *d* ascus (after Guilliermond)
Cryptococcus *e* vegetative cells
Candida *f* diagrammatic section of a colony on solid medium showing surface mass of yeast like cells (*g* details) and submerged clusters of budding cells connected by pseudomycelium (*h* details), *i* chlamydospores of *C albicans* (All except *f* × 650 approx)

Table IV

Differentiation of Candida Species

	Cand da albicans	*C trop cal s*	**C kruset*
Growth on Sabouraud s Agar	Creamy	Creamy	Flat and dry
Growth on Blood Agar	Medium-sized dull grey colony	Mycelial fringe to colony	Colonies irregular in size and shape
Growth on Cornmeal Agar	Mycelium†	Mycelium	Mycelium wi h blastospores like crossed sticks
	Chlamydospores†	No chlamydospores	No chlamydospores
Carbohydrate reactions			
Glucose	A(acid) and G(as)	AG	AG
Maltose	AG	AG	—
Sucrose	A	AG	—
Lactose	—	—	—

(*Adapted from* Martin, Jones Yao and Lee (1937) p 117)

* This species s possibly an imperfect state of *P h a* (Saccharomycetaceae) and would be better classified in the genus *Mycoderma* (Mackinnon and Artagaveytia Allende 1943)

† See Fig 2, *h l*

The taxonomy and nomenclature of pathogenic yeasts, and of the genus *Candida* in particular, is complex. Diddens and Lodder (1942) cite 87 synonyms for *C. albicans*. The problem has been much clarified during recent years and it need only be mentioned that, unfortunately, there are serious technical objections to the generic name *Candida* which may have to be replaced by an older and less ambiguous name. It was customary for medical writers to classify these yeast-like fungi as *Monilia* (hence the disease name [illegible] a genus properly used for the fungi [illegible] as since 1929 [illegible] b [illegible] " [illegible] decision of an International B[illegible] this were found to be possible and desirable.

The Problem of Pathogenic Status

The best known, and medically the most important, of the mycelial yeasts is *Candida albicans*. This fungus was recognized by Gruby in 1842 as the cause of thrush, a disease of the buccal cavity characterized by a white fungal growth over the mucous membrane lining. In addition, it is responsible for paronychia (an inflammatory infection of the nail bed), cutaneous infections (sometimes generalized but more usually confined to the interdigital spaces and to the axillae), vaginitis, pathological conditions of the digestive tract, and disease of the lungs. All these conditions are expressions of the disease complex known as moniliasis in which other species of *Candida* may also be involved. According to Mackinnon (1946), *C. albicans* appears to be the only cause of thrush and interdigital erosion while *C. tropicalis* often replaces *C. albicans* as the cause of moniliasis of the lungs and paronychia. Several other pathogenic species are recognized by the same author who regards *C. krusei* and *C. chalmersii* as two common saprophytes which are frequently found contaminating isolates of pathogenic species.

Vaginitis caused by *C. albicans* is most prevalent among pregnant women, especially during the three months preceding labour, probably due to an increase of glycogen in the vaginal mucosa, there being spontaneous cure after delivery. Babies may contract thrush at birth from such infected mothers with the result that the disease occasionally becomes epidemic in a maternity ward. The demonstration of the fungus nature of the patches of thrush and the death of affected children leaves little doubt of the mycotic origin of the disease and the virulence of the pathogen. Similarly, the microscopic and

cultural demonstration of a species of *Candida* from a typical case of paronychia leaves little doubt that the fungus is present as a primary pathogen Under other circumstances, however, the status of the fungus may be far from clear and for other conditions the role of the fungus is often, if not usually, uncertain

During the nineteen-twenties it was thought that *Monilia psilosis* (now regarded as identical with *C albicans*) played an important part in the etiology of tropical sprue since this fungus was repeatedly demonstrated in the stools of many affected persons Later work led to the conclusions that sprue is due to a nutritional deficiency and that the infection by species of *Candida* was only a secondary effect. It also became apparent that *C albicans* occurs in the faeces of about 15 per cent of normal individuals, whether from cities or country districts, and thus the demonstration of *Candida* in the faeces is no conclusive evidence of intestinal disease Similarly, the demonstration of *Candida* in sputum is very unreliable evidence on which to base a diagnosis of moniliasis of the respiratory tract

Budding cells are frequently seen in stained sputum smears These cells may have originated from the mouth Species of *Candida* were demonstrated by Epstein in 1924 (*see* Skinner, 1947) in the mouths of 54 per cent of infants 2–6 weeks old, in 46 5 per cent of those up to one year of age, and in 38 5 per cent of the 1–6 year group, whilst Todd (1937) found *C albicans* in 14 per cent of 1,000 normal individuals (7 0 per cent mouth and throat, 3 1 per cent mouth only, 3 9 per cent throat only), the incidence in females (18 2 per cent) being higher than in males (9 3 per cent) The incidence of yeasts may exceed even this level, particularly in persons with partial or complete dentures for whom Knighton (1939) found the incidence of all yeasts to be 61 per cent in contrast to 32 per cent in a longer series of mouths without dentures (It is interesting to note that the incidence of *C albicans* in the last two series was approximately constant at 24 per cent and 22 per [illegible] are

[illegible]

which they can be demonstrated and their number must then be taken into account The number may mislead, for *Candida* species are able to multiply with great rapidity in sputum at laboratory temperature and so if the sample cannot be cultured immediately it is essential that it should be stored in the refrigerator Even if budding cells are demonstrated in a succession of sputum samples and evidence for an oral

origin can be discounted a diagnosis of moniliasis of the respiratory tract is not justified because it is now well established that *Candida* species are frequently present as secondary invaders, particularly in sufferers from tuberculosis In a series of nine surveys involving the examination of nearly four thousand samples of sputum from cases in which tuberculosis was known or suspected, the percentage of specimens in which *Candida* was demonstrated ranged from 8 5–75, 9–36 per cent yielding *C albicans* (Skinner, 1947, Table I) If tuberculosis or other infection cannot be demonstrated and if X-ray examination reveals a pulmonary defect, then the repeated isolation of *Candida* gives greater justification for a diagnosis of moniliasis, this opinion may be supported by serological evidence (*see* p 70) but it is recovery after the application of therapeutic measures against moniliasis which affords final proof that the diagnosis was correct

Two types of lung involvement by species of *Candida* have been distinguished. In the one, bronchopulmonary moniliasis or "bronchomoniliasis," which was first recognized by Castellani about 1005, infection is confined to the bronchi, in the other, for which the term "pulmonary moniliasis" is reserved by Conant *et al* (1945), the parenchyma of the lung is involved The first is the commoner and milder of the two The chief symptom is a cough and, in 1906, Castellani diagnosed "tea tasters' cough" in Ceylon as a form of bronchomoniliasis, the infection being introduced in the tea dust which entered the nasal cavities when the quality of dry tea was tested by its smell X-ray examination usually shows little more than a non-specific type of peribronchial thickening in contrast to the heavier X-ray shadows of pulmonary moniliasis which resemble those of broncho-pneumonia (Conant *et al*, loc cit.)

These two categories of moniliasis of the lungs are not, however, universally accepted Shrewsbury (1936) in the light of an extensive examination of the occurrence of *Candida* in tuberculous sputum and in oral, nasal, and throat swabs from healthy babies and adults, while recognizing that secondary thrush of the bronchi does occur, came to the conclusion that—

> A critical review of the literature of bronchomoniliasis does not reveal any convincing reasons for the retention of the name in medical nosology A primary, independent disease should exhibit some distinctive clinical and pathological characters, bronchomoniliasis exhibits none that cannot better be referred to some other recognized disease

Even if this conclusion is rather extreme, it still finds supporters.

who was found after death to have been suffering from a pancreatic cancer

The recent recognition of the occurrence of moniliasis as a secondary effect following the therapeutic use of antibiotics has focused attention on the possible effect of competition between the bacterial flora and *Candida* in limiting the growth of the latter Harris (1950) drew attention to the possibility that *C albicans* normally present in the bowel may overgrow and perhaps increase in virulence following the destruction of the intestinal bacterial flora by the action of aureomycin or chloramphenicol (Chloromycetin*) and then invade tissues, the resistance of which may have been lowered by the effects of riboflavin or vitamin B complex deficiency More recently, Woods, Manning, and Patterson (1951) have given particulars of twenty-five cases of clinical moniliasis following the therapeutic use of penicillin, aureomycin, or chloramphenicol In twenty patients the oropharynx was involved 24–72 hours after successful treatment of a sore throat with an antibiotic. Three of the other cases were intestinal and the remaining two were of bronchomoniliasis. *In vitro* studies by these authors showed [illegible] no effect on the [illegible] nutriment in the substrate which allowed the moniliasis to develop They also tentatively concluded that treatment with vitamin B seemed to have some therapeutic value

There is little evidence of any consistent differences in pathogenicity between "saprophytic" and "parasitic" isolates of *C albicans* and Rothman cites an interesting observation that *C albicans* from a patient with monilial granuloma in which the oral mucosa, scalp, face, and finger nails were involved, caused only the usual and easily cured superficial intertriginous moniliasis in an individual of normal susceptibility

It should now be clear that the right interpretation of fungi found in sputum or associated with pulmonary disorders is a matter for experienced judgment Neither the mycologist nor the clinician alone is usually in a position to come to a correct decision. A mycologist who has isolated a potentially pathogenic fungus is as wrong to assume that it was present as a primary pathogen without full knowledge of the case as is the clinician to attribute disease to any fungus which may be reported as having been isolated from pathological material without

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considering the normal distribution of the organism in question On the other hand, in the opinion of David T Smith (1946, p 3), "mycotic infections occur with sufficient frequency to justify their being considered in the differential diagnosis of every difficult and complicated pulmonary and systematic infection"

References

Mycologists are referred to the mongraphs by Diddens and Lodder (1942) and Mackinnon (1946) for details of *Candida* and other yeasts or yeast-like fungi of medical importance and to the paper by Conant (1950) for the technique of sputum examination Useful clinical accounts of pulmonary mycoses are given by D T Smith (1947), Conant *et al* (1945), and Almeida and Silva Lacaz (1942) The valuable review of the genus *Candida* by Skinner (1947) should be consulted

here, as infection of the foot is the primary interest, the condition i designated by the original name

The granules in Madura foot caused by actinomycetes are usuall small and may be white or yellow (*Nocardia madurae*) or red (*Streptomyces somaliensis S pelletieri*) They are entirely composed of fin radiating hyphae and the peripheral hyphal endings may be club-like The actinomycetes usually associated with Madura foot are aerobi species That is, they are probably normal members of the flora of th soil and decaying vegetable matter Occasionally an anaerobic actinomycete is involved In current usage, actinomycosis in its variou manifestations is the disease caused by anaerobic species which are only found in association with living organisms Some authors woulc

Table V

REPRESENTATIVE ORGANISMS CAUSING "MADURA FOOT

Organism	*Geographical Distribution*	*Colour of Gra n*
ACTINOMYCETES		
Actinomycoses (Chalmers and Archibald) Mycetomas (Pinoy)		
Actinomycetaceae (anaerobic)		
Actinomyces		
A israeli	Brazil and elsewhere	yellowish
Nocardiaceae (aerobic)		
YELLOW GRAIN MYCETOMAS		
Nocardia		
N asteroides (Eppinger) Blanchard	Europe Brazil, Argentina *Philippines*	yellow
N bahiensis da Silva	Brazil	yellow
N convoluta Chalmers and Christopherson	Sudan (Khartoum)	orange
N madurae (Vincent) Blanchard	Africa (Abyssinia Algeria Egypt Senegal Somaliland) America (Cuba Argentina) Asia (India) Europe (Cyprus Greece)	white
N mexicana (Boyd and Cutchford) Ota	U S.A. (Los Angeles)	white-yellowish
N poncei Verdun		yellow
N transvalensis Pijper and Pullinger	S. Africa	whitish
Streptomycetaceae (aerobic)		
RED GRAIN MYCETOMAS		
Streptomyces		
S africanus (Pijper and Pullinger) Waks and Henrici	S Africa	red
S pelletieri (Laveran) Waks and Henrici	Egypt Senegal India Brazil	red
S somaliensis (Brumpt) Waks and Henrici	Somaliland, Sudan C. Africa	reddish-yellow

TABLE V—(contd)

Organism	Geographical Distribution	Colour of Grain
FUNGI IMPERFECTI		
Maduromycoses (Chalmers and Archibald), True Mycetomas (Pinoy)		
Moniliales		
BLACK GRAIN MYCETOMAS		
Glenospora		
G. clapieri Catanei	Algeria	black
G. khartoumensis Chalmers and Archibald	Sudan	black
G. semoni Chalmers and Archibald	France ("Indian soldier")	black
Madurella		
M. americana Gammel	U.S.A.	black
M. boroi Brumpt	Germany	black
M. grisea Mackinnon, Ferrada-Urzua, and Montemayor	Paraguay, Argentina	black
M. ikedai Gammel	U.S.A.	black
M. lackawanna Hanner and Zurett	U.S.A.	black
M. mycetomi Brumpt	Arabia, Senegal, Brazil	black
M. oswaldoi Horta	Brazil	black
M. ramiroi da Silva	Brazil	black
M. tabarkae Blanc and Brun	Tunis	black
M. tozeuri (Nicolle and Pinoy) Pinoy	Tunis	black
Penicillium		
P. mycetogenum Mantelli and Negri	Italy	black
Phialophora		
P. jeanselmei (Langeron) Emmons	Martinique U.S.A. (hand)	black
WHITE GRAIN MYCETOMAS		
Aspergillus		
A. bouffardi Brumpt		white
A. nidulans (Eidam) Winter	U.S.A.	white
A. nidulans var. *nicollei* Pinoy	Tunis	white
Cephalosporium		
C. falciforme Carrion	Puerto Rico	white
C. recifei Leão and Lobo	Brazil	yellow-white
Indiella		
I. brumpti da Silva	Brazil	white
I. mansoni Brumpt	Native of India and a Chinaman	white
I. reynieri Brumpt	France, Greece	white
Monosporium		
M. apiospermum Saccardo (imperfect state of *Allescheria boydii* Shear)	Italy, Algeria, Brazil, U.S.A.	white

(*Data from* Gammel (1927), Mackinnon, Ferrada, and Montemayor (1949) *etc.*)

to treatment Other disease complexes differ from monilial paronychia in that the response to treatment is conditioned by the pathogen involved. Tinea capitis in children caused by *Microsporum canis*, for example, has a more favourable prognosis than the similar condition caused by *M audouini* (*see* p 13) and tinea pedis caused by *Trichophyton rubrum* is usually more intractible than that caused by *T interdigitale* As far as is known, both Madura foot and chromoblastomycosis resemble paronychia in that infections by different fungi give rise to a well-defined clinical picture and call for like treatment The most interesting etiological difference between these two conditions is that whilst chromoblastomycosis is caused by a small group of related fungi, several taxonomically unrelated groups of related fungi (*see* Table V) are responsible for Madura foot

Most pathogenic fungi are mycelial in culture In the parasitic state three rather different types of growth may be distinguished The parasite may produce mycelium freely as when the outer layers of the skin become riddled with the mycelium of a dermatophyte Similarly, the pseudomycelium of *Candida* may at times grow freely into the tissues of the intestinal wall or of the tonsils, when, as for some dermatophytes, this invasive mycelium causes little local reaction by the tissues Usually the parasitic growth is more restricted when the pathogen appears as non-mycelial units or small groups of cells or the mycelial growth may develop as granules within mycetomas as in Madura foot It may be noted that the two examples of parasitic mycelial growth cited are both of endogenous pathogens, that is of fungi normally found associated only with animals and well adapted for parasitic existence The more restricted type of growth is typical of exogenous pathogens for which parasitism, even in fungi only known associated with disease, is possibly only incidental The unicellular parasitic habit is often characterized by yeast-like budding cells These are dealt with in more detail in the next chapter (*see* p 53) Here the mycetoma will be briefly considered

Since Carter's time, the mycotic granules of Madura foot have been often compared with the sclerotia which are a common feature of many different fungi This analogy cannot be maintained, for although the resemblance of a granule from a black grain mycetoma to a sclerotium may be striking the similarity is only superficial A sclerotium is a hard compacted mass of plectenchymatous tissue, usually dark in colour, and from a millimetre or so to more than 25 cm in diameter (*see* Fig 7) Internally there is usually a differentiation into a central region,

in which it may or may not be possible to distinguish the constituent hyphal elements, and a cortex of isodiametric cells of which the [illegible]

produced at the expense of the reserve materials accumulated within the sclerotium A granule from Madura foot contains no special reserve materials, it does not give rise to fruiting structures, and it is not a development from a colony It is in fact a whole colony and a closer analogy would be to spherical mould colonies which develop submerged in a solid medium or in shaken liquid cultures

Most, but not all, fungi when grown in a suitably aerated, liquid-culture medium, which is kept in constant motion on a shaking machine, develop as more or less spherical colonies The size of the colony, its colour, and its general morphology are often characteristic for the species (*see* Burkholder and Sinnott, 1945) and when the internal structure is elucidated by microscopical examination, the radiating arrangement and sometimes definite concentric zoning of the hyphal constituents recall a Madura foot granule Most moulds are not sensitive to gravity and the disc-like surface growth in a stationary culture is determined by the unilateral effects of the nutritional gradients and the aeration The development of a granule of a pathogenic fungus in tissue might be merely the result of growth in a uniform three-dimensional environment Such a hypothesis could explain the development of a granule in a pocket of pus but it would hardly suffice when the immediate environment is living tissues and the club-like endings to the peripheral hyphae of an actinomycotic granule probably indicate a host-parasite interaction In this connexion it is interesting to note that in an experimental shaken culture, whilst the mechanical effect of one colony rubbing against another is possibly a factor in determining the morphology, the tips of the peripheral hyphae are undifferentiated, and on a colony coming to rest they grow out radially

Attention has already been drawn to the similarity between an actinomycotic granule from Madura foot and one from a lesion of actinomycosis (*Actinomyces israeli*) (Plate VI (2, 3)) The fungus granule of Madura foot also has its counterpart in *Aspergillus fumigatus* infection of the lung in which the parasite may develop as a compact growth of radiating hyphae with deeply staining tips Both Henrici

(1940) and Almeida (1946) have drawn attention to instances of the development of "asteroid" or "actinomycetoid" forms of a number of other pathogens including *Sporotrichum schenckii*, *Coccidioides immitis*, and *Paracoccidioides brasiliensis* in which the normal cell becomes surrounded by a microscopic fringe of radiating processes. The most probable explanation of these abnormal outgrowths is that they are a defence reaction of the fungus to the host and Henrici (1940) discussed the possible bearings on this phenomenon of the two phases of the infection of experimental animals by *A. fumigatus*. The first result of infection is the development of well-defined abscesses, especially in the kidney; after a week to ten days the lesions become caseous, macrophages accumulate and may fuse to form giant cells, and so the lesion becomes converted into a tubercle. In the abcess the mycelium is abundant and basophilic. After caseation the mycelium becomes pale and acidophilic, much of it disintegrates but some grows out again as the peculiar actinomycetoid form. At seven to ten days the skin becomes hypersensitive to the pathogen and Henrici interprets the actinomycetoid form as the result of an interaction of the parasite with sensitized tissue.

Carrion and Silva (in Nickerson, 1947) figure short germ tubes from the cells of *Phialophora* species in tissue but the reason for the lack of mycelial development in chromoblastomycosis is unknown. (It might be interesting to compare the growth of the causal agents of Madura foot and chromoblastomycosis in shaken culture.) The reason for the success of these species as pathogens is also obscure. Saprophytic growth in soil or on plant debris from which the fungi could be introduced into a traumatic lesion on the foot would appear to be a prerequisite but why some and not other fungi are constantly associated with these conditions is still unknown. The physiological studies of fungi from Madura foot have so far provided no clue. Some species of *Madurella* causing black grain mycetomas have an optimum temperature for growth in the neighbourhood of 37°C but for *M. grisea* the optimum is 30°C and for other fungi is as low as 25°C. It may be that the ability to make growth under unfavourable conditions is of greater importance than is the ability to make optimum growth under conditions prevailing in the living host.

References

The literature of Madura foot is extensive and confused and the disease badly needs monographic treatment. The papers by Gammel

(1927), Chalmers and Archibald (1916), and Musgrave and Clegg (1907) all have long bibliographies, whilst the important paper by Brumpt (1906) must still be consulted. Mackinnon, Ferrada, and Montemayor (1949) have recently dealt with the black maduromycoses of South America

The papers by Carrion (particularly Carrion and Silva in Nickerson, 1947 and Carrion, 1950) should be consulted for details of chromoblastomycosis

CHAPTER V

SYSTEMIC MYCOSES

The Problems of Dimorphism and the Source of Infection

THE topics dealt with in this chapter are illustrated by a consideration of six important systemic mycoses and, as a background for more detailed discussion, the general characteristics of these diseases are first summarized

Coccidioidomycosis (*Coccidioides immitis*), which has a focus in the warm arid regions of the United States, was first described in Argentina in 1892 by Posadas and Wernicke who attributed the disease to a protozoan Four years later Rixford and Gilchrist described two cases in Southern California and proposed the name *Coccidioides immitis* for the pathogen which was cultured and proved to be a fungus by their fellow countrymen Ophuls and Moffit in 1900 The early cases, in which mortality was high, showed great clinical diversity, the lungs, bones, skin and subcutaneous tissues, and the central nervous system being variously involved In 1937, however, evidence was obtained that a mild respiratory disease prevalent in the same area and known as "Valley fever" (after the San Joaquin Valley, California), "desert rheumatism," or "the bumps" was the primary form of coccidioidal granuloma, as coccidioidomycosis was then called. Subsequent investigation showed that only about 0 2 per cent of cases of the primary form in white males (and 3 5 per cent in negroes) develop into disseminated infections (*See* Emmons, 1942, Emmons in Nickerson, 1947)

Torulosis (*Cryptococcus neoformans*) is characteristically a disease of the central nervous system although the lungs, and less frequently the skin (*see* Cawley, Grekin, and Curtis, 1950), may also be involved Most frequently the disease takes the form of a meningitis or meningo-encephalitis, when it can easily be confused with tubercular and other forms of subacute chronic meningitis There are published records of about 150 cases from all parts of the world and the frequency with which the diagnosis has been made after death suggests that the disease has a higher incidence than these records indicate "If any treatment favourably influences the course of torulosis it is unknown" according

to Cox and Tolhurst (1946) whose monograph and the review by Lodder and De Minjer (in Nickerson 1947) may be consulted for further details

Sporotrichosis (*Sporotrichum schencki*) which has recently been reviewed by Norden (1951) is widely distributed Unlike the two [illegible]

[illegible]

forearm (Plate VII (3)) and this is followed by a succession of gummata each similar to the primary lesion at intervals along a thickened lymphatic vessel Sporotrichosis is rarely fatal and responds well to iodide therapy

North American Blastomycosis (*Blastomyces dermatitidis*) This disease as its name implies is endemic to North America where it is most prevalent in the eastern half of the United States Two clinical patterns are exhibited systemic and cutaneous and the granulomatous lesions of the latter rarely give rise to the disseminated form in which infection of the bones particularly the vertebrae and ribs is common Systemic blastomycosis is usually fatal whilst untreated cutaneous lesions may persist for many years Primary pulmonary infections are common and are liable to be mistaken for tuberculosis

Histoplasmosis (*Histoplasma capsulatum*) Like *Coccid oides imm tis H stoplasma capsulatum* was first thought to be a protozoan by Darling who described three fatal cases in Panama in 1905–06 The next record was from Minnesota twenty years later since when many more cases have been encountered in the United States where histoplasmosis is probably endemic and occasionally in the British Isles and other parts of the world The fungus invades the reticulo-endothelial system and gives rise to fever emaciation anemia and leucopenia which are often accompanied by splenomegaly and ulceration The prognosis is grave. Death frequently results from within a few weeks to under a year although the disease may take a prolonged chronic course and spontaneous recovery has been recorded

It should, however be mentioned that recent surveys have revealed many persons in certain parts of the United States who while showing healed pulmonary lesions give no reaction to the tuberculin test but are histoplasmin sensitive (*see* p 72) This suggests that histoplasmosis *exists in an unrecognized perhaps subclinical form in these areas*

South American Blastomycosis (*Paracoccid oides brasiliensis*) is limited to South America and is particularly prevalent in the state of

3–4μ in diameter on hyphae near septa and slightly larger, (4–5μ) spherical to pyriform on laterally developed conidiophores Old cultures show numerous chlamydospores 7–12μ in diameter Experimental infection of animals effected by inoculation with the mycelial growth results in a return to the yeast phase A primary culture at first tends to be yeast-like and the yeast phase may be induced at will *in vitro* by growing the fungus on blood agar or nutrient glucose-agar at 37°C By this means, thick-walled budding cells indistinguishable from those of the parasitic phase result Similarly, by modifying the environment Lutz and Splendore in 1908 induced the yeast phase of *S schencki in vitro* by growth on inspissated human serum Since then other media such as Francis' glucose cystine agar or brain-heart infusion agar have been shown to be equally suitable provided the incubation temperature is raised to 37°C The yeast form is also pathogenic for susceptible animals and is usually the most convenient inoculum for experimental purposes

[illegible]

[illegible] indistinguishable from *B dermatitidis* when diagnosis is dependent on cultural studies but if multiple buds are observed a diagnosis can be made, for these structures are unknown elsewhere *in vivo* In section, the spherical parent cell first shows minute dot-like projections from the periphery These increase in size and are finally pinched off so as to lie free in the tissue surrounding the mother cell which frequently collapses In culture at laboratory temperature on Sabouraud's agar growth is slow and a rather glabrous colony showing tufts of white aerial mycelium results Dowding has drawn attention to the tendency for the mycelium to grow as three-dimensional network and she designates the small terminal or intercalary conidia (1–4μ diameter) microconidia At 37°C on nutrient glucose agar the growth form is that of a multiple budding yeast *In some strains* the budding cells tend to remain massed together (as in *P cerebriforme*) while in others they break apart to give the free-yeast form If a series of sections be cut through a multiple

which sometimes exhibit budding. In culture on Sabouraud's agar at [illegible]

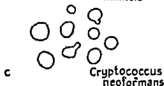
On blood agar at 37°C the moist and yeast-like growth is composed of small budding cells like those seen in animal tissue Recently, Dowding observed that in the experimental mycelium-yeast transformation the tuberculations of the large thick-walled spores increase in size and are budded off in a similar manner to the multiple buds of *P. brasiliensis* She therefore interprets the tuberculated spore of *Histoplasma* and the multiple budding cell of *Paracoccidioides* as being homologous, and regards them both as macroconidia and the buds as secondary conidia, the smaller conidia produced in culture being microconidia Brandt (1950) states that when spores of *Histoplasma* are injected into an experimental animal, endogenously formed yeast cells are liberated into the tissues, that is, the spore functions as a sporangium Such a development has not been observed by Dowding who has stated (1951, *in litt*) that in her hands Feulgen stain reveals nuclei in the spores but not in the spore tuberculations and that this, together with the bizarre shapes the tuberculations sometimes take, suggests that they may be mucilaginous exudations She still, however, believes that given the right conditions, the tuberculations become yeast cells The relationships of the growth forms of these fungi are illustrated in Fig 3

The interpretation of dimorphism is a matter for speculation The yeast-like, or non-mycelial, form is characteristic of the parasitic phase and the suggestion has been made by Duncan (1948) that this habit may be a special adaptation for parasitism If so, the ease with which the transformation from a saprophytic to a parasitic mode of life is effected [illegible] man than is supposed or there may be an animal reservoir, which for coccidioidomycosis and histoplasmosis is not unlikely (*see* below) Also, it may be noted, *Histoplasma farciminosum*, which causes epizootic lymphangitis in horses, is a dimorphic fungus and that dimorphism is common among entomogenous fungi of the Entomophthorales Alternatively, dimorphism may be an inherent character of certain

SAPROPHYTIC PHASE PARASITIC PHASE

25° C 37° C

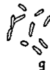


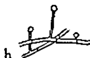


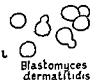




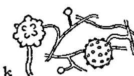


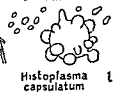


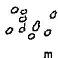












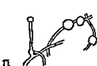






FIG 3 DIMORPHISM

fungi, a hypothesis in line with the generally accepted view that filamentous forms have been derived from unicellular ancestors and one which can be supported by experimental evidence A typical mycelial fungus such as the common saprophyte *Mucor racemosus* if grown submerged in sugar solution forms chains of budding cells and like brewer's yeast, gives rise to alcohol Plant pathogenic fungi such

brooms in a number of higher plants Additional evidence is forthcoming from the recent investigations by Nickerson on the mechanism of the mycelial-yeast conversion That author believes the formation of filamentous elements from a yeast cell to be due to the selective inhibition of growth (which he defines as an irreversible increase in volume), and experimental evidence indicates that for *Candida* compounds such as cysteine and glutathione, which contain sulphydral (-SH) groups promote cell division and hence suppress mycelium formation, whilst cobaltous ions by the inhibition of cell division have the reverse effect (Nickerson 1948) In *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis* the interconversions appear to be dependent on temperature of incubation only (Nickerson and Edwards, 1949) while Salvin (1947b) found that in *Sporotrichum schenckii* the maximum quantity of budding cells (and occasional multiple budding) and the minimum of mycelium required a temperature of 37°C, 0 15–0 2 per cent agar, pH 8 2, and an increase of carbon dioxide tension to between 60 and 80 per cent. Such evidence as this appears to favour the view that dimorphism is a widely-distributed phenomenon among fungi and that it is not peculiar to fungi causing systemic mycoses in man. As for granule formation in mycetoma which was discussed in the

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KEY TO FIG 3

cells of parasitic phase

Blastomyces dermatitidis Mycelial (*h*) and yeast () phases in culture *j* yeast cells of parasitic phase *note* double-contoured wall.

Histoplasma capsulatum *k*, macro- and micro-conidia *l* tuberculations of macroconidium giving rise to budding cells (after Dowding 1948

le budding

(Budding

preceding chapter, widely differing fungi are potentially dimorphic (it would be interesting to ascertain whether the saprophytic species of *Sporotrichum*, for example, possess this ability) and those able to function as pathogens are those which in addition are able to tolerate the conditions prevailing in the animal body

Source of Infection

As previously noted (p 5), mycoses may have an endogenous or an exogenous origin. In exogenous diseases the infection may be derived from another man, from an animal, or from some other source. All the systemic mycoses under consideration *are believed to be exogenous* and there is no evidence that they are normally contracted from human *sources*

In Table VI the incidence of these diseases is summarized according to age, sex, and occupation This tabulation suggests that men are more often attacked than women, perhaps because men are more frequently exposed to the hazard of infection Another suggestive relationship is the frequency of sporotrichosis among those engaged in agriculture and horticulture and as in this disease the primary lesion is *usually on the hand or forearm and can be traced to injury* by a thorn or other plant material, strong clues to the normal habitat of the pathogen are provided

A very interesting contribution to the epidemiology of sporotrichosis was made by Mackinnon (1949) who was able to ascertain the date of infection in 32 of a series of 46 cases of sporotrichosis which he studied in Uruguay between 1939 and 1948 Twenty-six patients were found [illegible]

pathogen on its natural habitat was promoted and the chance of infection increased An examination of the weather records of periods when epidemic infections occurred suggested that a relative humidity [illegible]

Mackinnon claims to have forecast the small epidemic outbreak of five cases which occurred in April, 1947

Another species of *Sporotrichum*, *S poae*, causes bud rot in carnations and silver top disease in June grass, and Benham and Keston (1932) showed that *S schencki*, also is pathogenic for plants These workers

Table VI

RELATION OF AGE, SEX, AND OCCUPATION TO INCIDENCE OF SYSTEMIC MYCOSES

Disease	Age		Sex Ratio (M = male, F = female)	Occupations with highest incidence
	Range	Highest incidence		
Coccidioidomycosis (*Coccid oides imm tis*)	0·25–70	25–55	Primary M = F Progressive M > F	Labourers (most frequent in pigmented races)
Torulosis (*Cryptococcus neoformans*)	10–70	40–60	2M 1F	—
Sporotrichosis (*Sporotrichum schencki*)	1 3–71	Average age 29	M > F (46M 1F in Uruguay, Mackinnon, 1949)	Farmers, horticulturalists, labourers
North American Blastomycosis (*Blastomyces dermati dis*)	0·5–80	20–40	9M 1F	Poorer classes
Histoplasmosis (*Histoplasma capsulatum*)	0·25–70	Children under 13, 28 per cent	Children 2M 1F Adults 3M 1F	—
South American Blastomycosis (*Paracoccidioides brasiliensis*)	11–80	21–30	11 5M 1F	Manual labourers

(*Data for South American Blastomycosis from* Almeida (1939) *for other diseases from* Conant *et al* (1945))

successfully infected carnation buds, a living rose bud, and detached thorns and berries of the barberry with *S schencki* Positive results were also obtained in carnation with other species of *Sporotrichum* from man, with *S poae*, and with an isolate of *S purinosum* from soil The pathogenicity of *S schencki* for rats was shown to be unaffected by passage through carnation and whilst rats were successfully infected with *S purinosum*, *S poae* proved to be non-pathogenic for these animals

An outbreak of sporotrichosis in the gold mines of the Transvaal during 1941–43 provides one of the most spectacular outbreaks of mycotic disease ever recorded In a little more than two years there were 2,825 cases of sporotrichosis among miners at the Ventersport and the Consolidated Main Reef mines (*see Sporotrichosis* . , 1947) Sixty per cent of the lesions occurred on the hands and arms The source of infection was traced to a saprophytic growth of the fungus on wooden mine props and the epidemic was checked by spraying the props with [illegible]

and extremely wet It may also be noted that the maximum incidence at the Ventersport mine was during January and March, the months of [illegible]

chlamydospores and the port of entry is usually the lungs In endemic areas its incidence is most noticeable among migrant or newly-resident adults and among children of school age, and there is a marked association of cases of the disease and previous exposure to dust storms and agricultural dust The seasonal incidence of coccidioidomycosis is the reverse of that of sporotrichosis It is highest in California during the dry months of June to September and falls when the autumn rains begin The incidence among troops stationed in the San Joaquin Valley was reduced by one third to a half by grassing airfields and by spraying athletic grounds with mineral oil (Smith, Beard, Rosenberger, and Whiting, 1946) These facts would appear to implicate the soil and *Coccidioides immitis* has been isolated from soil in California and Arizona on several occasions In one small outbreak involving seven of fourteen university students who made a field trip to the San Joaquin valley proof that the infection originated from soil was almost

conclusive All the seven students who contracted the disease, and two others who had apparently been immunized by a previous infection, were subjected to a very heavy dust exposure when digging out a rattlesnake which had entered a ground squirrel's hole and subsequently *C immitis* was isolated from soil collected at the site of the incident (Davis, Smith and Smith, 1942) The ecological relationship of the fungus to the soil is, however, far from clear The nutritional requirements of *C immitis* are not exacting (*see* p 6) and if it was a common saprophyte on plant debris its very restricted geographical distribution would be difficult to explain A possible explanation of the localization of the disease would be an association with an animal host of limited distribution Infected sheep cattle, and dogs have been reported in endemic areas but the sporadic incidence suggests that these animals, like man, are only accidentally infected and do not constitute a reser-

found that 80 per cent of the pocket mice (*Perognathus* spp) had a pulmonary mycosis due to one or both of two fungi, *C immitis* and the related *Haplosporangium parvum* In 124 specimens of *Perognathus*, *C immitis* was found in 19 and *H parvum* in 95 The kangaroo rat (*Dipodomys merriami*) and the Harris ground squirrel (*Citellus harrisi*) were also frequently found to be infected On the other hand the white-footed mouse (*Peromyseus eremicus*) which is more abundant than the pocket mouse, and the grasshopper mouse (*Onychomys* spp) were rarely infected although living in burrows adjacent to pocket mice and in spite of the fact that *Peromyseus* is very susceptible to a fatal infection under experimental conditions The evidence indicated that the infection of these rodents is a chronic disease which has probably little effect on the population and Emmons comes to the tentative conclusion that the presence of *C immitis* in soil is due to contamination from infected rodents and that it is the distribution of appropriate species which limits the distribution of coccidioidomycosis

the same fungus in the beaver (*Castor canadensis*) (Erickson 1949)

The natural habitats of the pathogens causing the other four diseases are unknown and the data on the incidence of these mycoses gives little help *Cryptococcus neoformans* was originally isolated by Sanfelice

in 1895 from the surface and juice of a peach and found to be pathogenic for guinea-pigs Klein in 1901 isolated the fungus from milk but there is no evidence of man contracting infection from such sources Naturally-infected animals have been found, but here again there is no evidence that these infections are of any significance in the epidemiology of human torulosis Yeasts very similar to C *neoformans* are commonly found associated with man and some, such as *Torulopsis glabrata*, have been isolated only from human sources but Benham (1935) in an extensive series of animal inoculation experiments found the pathogenicity of all but C *neoformans* to be very doubtful It would appear that the non-mycelial yeasts from the point of view of pathogenicity, are a parallel series to the mycelial yeasts in which the factors which determine whether a normally saprophytic fungus becomes pathogenic are unknown

South American blastomycosis (*Paracoccidioides brasiliensis*) shows certain resemblances to coccidioidomycosis in symptomology (the two diseases have been at times confused), in being geographically localized, and in having a high incidence among manual labourers but no animal infections have been recorded and the source of infection is unknown North American blastomycosis (*Blastomyces dermatitidis*) is exceptional in that there are a few instances of spread by contagion from one individual to another, but this is very rare Naturally-infected dogs have been noted but these infections were probably only incidental In the only British example of this disease, in a man who was a wood-chopper by occupation the primary lesion was of the nail fold and systemic infection secondary (Dowling and Ellsworthy, 1925) This suggested that the infection might have been derived from wooden packing cases of imported American motor-cars since Stober (1914) in the United States, in addition to obtaining a profuse growth of *B dermatitidis* on bread, described and figured from the wooden walls of the house of one patient, a fungus which showed microscopical and cultural resemblances to the blastomycosis pathogen

The evidence for an animal reservoir for histoplasmosis is slightly stronger for there are a number of records of this disease in dogs In a recent test in the United States of 1,314 dogs with histoplasmin seven gave a positive skin reaction and in four of these dogs histoplasmosis was confirmed by cultural and microscopic methods (Cole, Prior and Saslaw, 1950) Emmons, Bell and Olsen (1947) made a survey of the rodent population in a rural district of Virginia where four human and three canine cases had been reported and if the results were not so

spectacular as those of the rodent survey in connexion with coccidioidomycosis they were at least suggestive, for *Histoplasma capsulatum* was isolated from one mouse (*Mus musculus*) and ten rats (*Rattus norvegicus*) in a collection of 1,620 animals

Raftery (1951) records finding organisms morphologically identified as *Histoplasma capsulatum* in more than 10 per cent of 436 appendixes surgically removed from children aged 16 and under during a ten year period and in a recent case the isolation of *H capsulatum* in culture Most of the children were suffering from an undiagnosed chronic disease and there was an unexplained high incidence of lymphoblastoma associated with the infections The high incidence of the disease [illegible]

calcifications in individuals who are tuberculin negative but histoplasmin positive suggests that man is exposed to a more general source of infection Thus, as for coccidioidomycosis, may be dust since *H capsulatum* has been isolated from soil samples from Virginia (Emmons, 1949) and Tennessee (Ajello and Zeidberg, 1951) Experimental inoculation of laboratory animals usually results in transient granulomata Do pulmonary calcifications indicate a parallel in man?

It is possible that for torulosis, and perhaps histoplasmosis, as for moniliasis (*see* p 35) infection is influenced by metabolic and other disturbances in the host. Approximately 10 per cent of torulosis meningitis cases have been associated with Hodgkin's disease and Cawley and Curtis (1948) have recorded four cases of histoplasmosis associated with lymphoblastoma (two with Hodgkin's disease, two with lymphatic leukemia) Such associations may be only accidental but if significant they may eventually throw light on the reasons for the incidental parasitism of an apparently natura saprophyte such as *C neoformans*

CHAPTER VI

SEROLOGY OF PATHOGENIC FUNGI

A Problem of Diagnosis

IMMUNITY or resistance shown by an organism to infection by a pathogen may be either *natural*, that is, an inherent character of each normal individual, or *acquired*, by chance or by design Natural immunity to fungal attack is widespread among both plants and animals Acquired immunity, on the other hand, is exhibited only by animals and so among mycologists only medical mycologists are usually familiar with the serological techniques used for the investigation of this phenomenon The theoretical aspects of serology are complex and the terminology esoteric but the underlying principles necessary for an understanding of the present chapter may be briefly outlined

Acquired immunity is dependent on the presence in the blood serum of substances detrimental to the invading organism. Such substances may result from an accidental infection (i e an attack of the disease) or they may be acquired artificially by the use of vaccines consisting of a sub-lethal dose of the pathogen, the dead pathogen, or products of the pathogen ("active immunity") or by the introduction of immune serum ("passive immunity") Active immunity may last for life, and when 'artificial' is normally a prophylactic treatment. Passive immunity is of shorter duration and is more often of therapeutic value The special substances present in immune serum are called *antibodies* and they result from the parenteral introduction into the animal body of other substances known as *antigens* An essential character of an antigen is that it reacts in some observable way with the antibody to which it gives rise When an antigen, such as a suspension of fungus spores, is mixed with serum containing the appropriate antibodies (the *antiserum*) there may be agglutination of the spores and the dilutions at which this reaction takes place is used to measure the titre (antibody concentration) of the antiserum. Similarly, mixing a non-particulate antigen with its antiserum may result in the formation of a precipitate

Agglutination and precipitation depend on a simple antigen-antibody interaction In lysis, another common antigen-antibody reaction, the

dissolution of the cells is dependent also on the presence of a thermolabile, non-specific substance or complex of substances present in the normal blood serum of all higher animals and known as *complement*. For example, the lysis of red cells of sheep's blood (the antigen) by sheep red-cell-immune serum (which contains the antibody) takes place only in the presence of complement, that is, in the presence of blood serum but not in the presence of serum which has been heated to 56°C for 30 minutes to destroy the complement

A general property of antigens after reacting (or being "sensitized") with their specific antibodies is an ability to "fix" complement and this ability is used as the basis of the sensitive *complement fixation test* for the detection of antigen-antibody reaction In this test the antigen, antibody (antiserum, after heating to remove complement), and complement (usually guinea-pig blood serum) are mixed and after a suitable interval red blood cells and haemolysin (red-cell-immune serum) added If antigen and antibody have reacted and complement fixation has occurred there is no change, if there has been no antigen-antibody reaction, lysis of the red cells results

The introduction of an antigen into an animal and the development of antibodies may result in the animal becoming hypersensitive to the antigen If, for example, ten days or so after a guinea-pig has been injected with egg albumin a second, and much smaller, injection of the same protein is made, death may result Usually, *allergic* reactions are less severe and give rise to conditions such as asthma and hay-fever (*see* Chapter VII) or to the localized reactions which form the basis of skin testing It must be emphasized that specific dermal sensitivity (such as that to trichophytin, histoplasmin, etc , *see* below) is not related to circulating antibodies and can be passively transferred to a non-sensitive subject only by skin-grafting

The [illegible]

fully understood

Before considering the use of serological methods for the detection of mycotic infections in man, attention must be drawn to the fact that a fungus is not a single antigen and so the antiserum prepared against any particular fungus will contain a number of different antibodies, [illegible]

a heterologous antiserum the antibodies for which it has the appropriate antigens and then testing the treated antiserum against the fungus for which it is the homologous antiserum, the presence of antigens common to the two organisms can be detected and by appropriate technique the degree of antigenic similarity can be given a quantitative value This method of cross-absorption has been used by mycologists to obtain evidence on which to base phylogenetic speculations, the presence of antigens in common being generally accepted as indicating a fundamental relationship

Trichophytin

During the first decade of the present century the Swiss worker Bloch found that guinea-pigs, after recovery from an experimenta infection with either *Trichophyton quinckeanum* or *T mentagrophytes*, were immune for as long as a year and a half to reinfection by the same fungus He found that this immunity, which was correlated with a hypersensitivity of the skin, could be induced only by cutaneous inoculation, subcutaneous or intraperitoneal injection of the living fungus or an extract of the fungus did not give rise to immunity or to skin sensitivity. Further, Bloch sensitized himself by inoculation with *T quinckeanum* and then grafted a piece of his skin and a piece of skin from a non-sensitized person over an ulcer on the leg of a third subject. Subsequent testing with a *Trichophyton* extract gave a positive reaction on the skin taken from Bloch but not on the skin of the control graft or on the skin of the patient.

It was Neisser and Plato in 1902 who first made an extract of a ringworm fungus, which they designated "trichophytin" and which on introduction into a patient suffering from ringworm caused a local erythematous response around the site of the injection and a generalized reaction accompanied by a rise in temperature Bloch found that the cutaneous response to trichophytin appeared a week or so after infection, an observation since confirmed by others who have shown that the skin sensitivity is sometimes retained for many years

Attempts to demonstrate agglutinins, precipitins, and complement-fixing antibodies in ringworm infections have failed This may be due to technical reasons, as Conant *et al* (1945) suggest, but it may be that as the infection is superficial the body cells do not produce detectable amounts of antibodies in the blood

Skin sensitivity is thus the only immunological response to infection which can be elicited The same effect may result naturally in generalized

signs in some infected persons, a condition first noted by Jadssohn in 1911 who described follicular eruptions in patients with kerion These eruptions or *dermatophytids*, which are sometimes distinguished as trichophytids and microsporids according to the genus of the dermatophyte involved, occur on the body, the feet and legs, and, most frequently, the hands, and they disappear spontaneously on the elimination of the primary fungus infection. It is unusual for a fungus to be isolated from a dermatophytid which is commonly thought to be an allergic response to the systemic spread of some metabolic product of the fungus, or perhaps of fungus spores or elements, for there are a number of records of the isolation of ringworm fungi from the blood

Recently, evidence has been obtained that sensitization to dermatophytes is one of the factors determining sensitivity to penicillin, the injection of which sometimes gives rise to a reaction simulating that to trichophytin (Peck, 1950)

Histoplasmin

The inability to obtain immune serum has hampered investigations designed to elucidate the processes underlying the empirical results of trichophytin testing Skin tests with extracts of other fungi, and particularly with coccidioidin (from *Coccidioides immitis*) and histoplasmin (from *Histoplasma capsulatum*), have given valuable results in the diagnosis and epidemiology of other diseases and as an example of modern methods of investigation and as a background for the interpretation of the results of serological diagnosis the salient features and results of a series of recent studies by Salvin on the serology of *H capsulatum* will be given (Salvin, 1947, 1949, 1950*b*, Salvin and Hottle, 1948*a*, 1948*b*)

The usual method for preparing a fungal extract for intradermal testing is to grow the fungus in liquid medium for a period of weeks or months, filter off the mycelial mat, and after the addition of 1 10,000 methiolate, to store the sterile filtrate in the refrigerator until needed The extract is suitably diluted with sterile saline before use

[illegible]

and neither sensitizes man or an experimental animal nor produces non-specific reactions, was that adopted by Salvin in his study of the factors which influence histoplasmin formation

The composition of this medium is—

l-Asparagine	14 0 g
Magnesium sulphate ($MgSO_4\ 7H_2O$)	1 5 g
Dipotassium phosphate (K_2HPO_4)	1 31 g
Sodium citrate ($Na_3C_6H_5O_7\ 5\frac{1}{2}H_2O$)	0 9 g
Ferric citrate (scales)	0 3 g
Glycerin	25 0 g
Glucose (the grade known as *Cerelose*)	10 0 g
Distilled water to	. 1 000 0 ml

Emmons and earlier workers estimated the titre of histoplasmin by the skin reaction in guinea-pigs inoculated with *H capsulatum* on a basis of the dilution of the histoplasmin and the diameter of the wheals obtained. Salvin developed an assay by means of a complement-fixation technique which he showed to be more accurate and to give reproducible results comparable to those of skin testing When this test was used to evaluate the effect of environment and of nutritive factors on the formation of histoplasmin it was found that the maximum yield of histoplasmin was obtained in the glucose-asparagine medium after eight to ten weeks, with an initial pH of between 7 and 8 a surface to volume ratio of near unity, and an incubation temperature of 25°C While a satisfactory titre of 16,000 was obtained with glucose, better yields were obtained by the replacement of glucose by similar amounts of cellobiose (64 000), dextrin (64 000), glycogen (128 000) or starch (128,000)

The next study was a comparative serological examination of the antigens derived from the yeast-like and mycelial phases of *H capsulatum* Seven antigens were prepared—

(*a*) *From the yeast phase* (*YP*)

1 "whole *YP* cells" killed by 0 5 per cent formalin and washed

2 "ground *YP* filtrate" obtained by grinding washed live cells in a ball mill and filtering through a Seitz filter

3 "*YP residue,*" *the residue from* 2

4 "*YP* broth filtrate" obtained by filtering a *YP* culture through a Mandler filter

(*b*) *From the mycelial phase* (*M*)

5 "ground *M* filtrate" prepared by grinding the mycelial mat in a ball mill and filtering

6 "ground *M* residue," the residue from 5

7 "*M* broth filtrate" (histoplasmin)

Antiserum against *H capsulatum* was obtained by infecting rabbits with the yeast phase and bleeding them at intervals In addition, a [illegible]

fixation test

In the precipitin tests, no antibodies were detected for the first eight days after infection of the rabbits With all antigens, peak titres were reached by the 16th or 20th day and the final disappearance of antibody titre varied from 49 days after infection with ground *M* filtrate as the antigen to 76 days with histoplasmin, the antigen with which the highest titre was obtained

In the complement-fixation tests with antiserum from infected rabbits only whole *YP* cells, *YP* residue, and ground *M* residue (the particulate antigens) fixed complement but with hyperimmune antiserum only ground *M* filtrate failed to fix complement, histoplasmin again giving much the highest titre

In cross absorption tests hyperimmune serum was absorbed with each of the antigens singly (with the exception of 3) and the subsequent complement-fixation tests showed that the *YP* cells and ground *M* residue removed all demonstrable complement-fixation antibodies and that the other four (all soluble antigens) failed to absorb the antibodies which fixed complement in the presence of whole *YP* cells, *YP* residue, or ground *M* residue

It was concluded from these results that the antigens elaborated by the mycelial phase and the yeast phase were similar and that histo- [illegible]

It is interesting to note that, whilst as already indicated the precipitin titre of serum from experimentally infected rabbits rose to a maximum and then fell to zero, the complement-fixation titre with yeast cells as the antigen attained its maximum two to three weeks later and then after a decrease remained constant for at least six months (see Fig 4) The precipitin titre maximum coincided with the maximum clinical symptoms and so the precipitin and complement-fixation tests provide a method for distinguishing the acute and chronic phases of histoplasmosis in rabbits

The final group of investigations related to the cross-reactions obtained between *H capsulatum* and heterologous sera The data from certain experiments are summarized in Table VII which clearly shows the antigenic inter-relatedness of H [illegible]
Coccidioides [illegible]
dermatitidis [illegible]
other three fungi *C immitis* and *C albicans* in this series of experiments

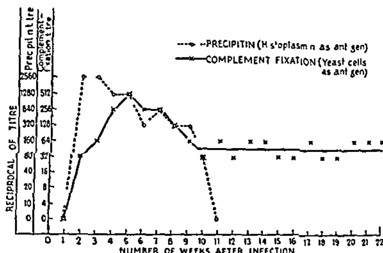


FIG 4 PRECIPITIN AND COMPLEMENT-FIXATION TITRES OF SERUM OF RABBITS EXPERIMENTALLY INFECTED WITH *Histoplasma capsulatum*
(*After Salvin and Hottle* 1948*a*)

reacted only with their homologous antisera although the antisera prepared against these two fungi reacted with certain heterologous antigens Other workers, according to Salvin (1949*b*), have reported that *C albicans* antigen fixed complement in the presence of *B dermatitidis* antiserum but no published record could be found of a cross-reaction between *C immitis* complement-fixing antigen and a heterologous antiserum

Cross absorption tests with the four antisera showed that for each antiserum all three heterologous antigens removed antibodies (that is, the complement-fixation titre of absorbed antiserum against the homologous antigen was lower than that of untreated antiserum against the same antigen) and that *B dermatitidis* had as great a combining power with anti-*H capsulatum* serum as had the homologous

antigen These results indicated that all four fungi had antigens in common and so each is theoretically capable of reacting with antisera prepared against the others It should, however, be noted that all the fungal antigens listed in Table VII gave the strongest reaction with their homologous sera

In addition, the 1939 experiments included cross-reaction tests with *Aspergillus fumigatus* and *Absidia corymbifera* antigens. No reactions

TABLE VII

CROSS-REACTIONS OF FUNGAL ANTIGENS

Antigen	Complement-fixation titres of antisera prepared against antigens (1)	(2)	(3)	(4)	(5)	(6)	(7)
1 *Histoplasma capsulatum*	**64**	16	16	16	*0*	*0*	*0*
2 *Blastomyces dermatitidis*	16	**32**	16	2	*0*	*0*	*0*
3 *Coccidioides immitis*	0	0	**32**	0	—	—	—
4 *Candida albicans*	0	0	0	**256, *128***	*64*	*0*	*0*
5 *Candida stellatoidea*	*0*	*0*	—	*64*	***1 024***	*0*	*0*
6 *Cryptococcus neoformans*	*0*	*0*	—	*0*	*0*	***2 000***	*0*
7 *Aspergillus fumigatus*	0	0	0	0	*0*	*0*	***128***
8 *Absidia corymbifera*	0	0	0	0	—	—	—

Data from Salvin, 1949b (titres in roman type) and Salvin, 1950b (titres in italics)
Titres of antisera against their homologous antigens in bold face type
—, No test.

were detected and neither could antisera of significant antibody titre [illegible]

antigen In the same series antisera were prepared against *Cryptococcus neoformans*, *Candida albicans* and *C stellatoidea* All these three reacted with their homologous antigens and the last two also reacted, but more weakly, with their respective heterologous *Candida* antigen. The results suggest that the more specific the antigen the greater its antibody-producing power (Table VII, antigens 4, 5, 6, 7) Attention may also be drawn to the antigenic dissimilarities of *A corymbifera* and *C immitis*, which are both phycomycetes, and the failure of *A fumigatus* to react with the antisera of other hyphomycetes such as *H capsulatum* and *B dermatitidis*

Serological Diagnosis

Specific cutaneous tests or serum reactions would be convenient, simple, and rapid means for the recognition of mycoses and consequently serological diagnosis receives perennial attention Different workers frequently prepare their diagnostic antigens by different methods, they use these preparations in different ways, and the mycological data are often inadequate for reasons beyond the investigator's control so that, as might be expected, many conflicting results have been reported The large and confused literature is difficult to summarize and here it is only possible to indicate certain established facts and to give examples of representative uses of the method

Infection by a pathogenic fungus usually results in skin hypersensitivity while the development of demonstrable antibodies in the blood is of less frequent occurrence Cutaneous testing has, therefore, predominated The chief interest in the trichophytin test, the first skin test to be devised, has been in its use for the rapid diagnosis of infection by a ringworm fungus The evidence so obtained clearly indicates that infection by a dermatophyte usually, but not always, results in skin sensitivity, that patients showing dermatophytids give a positive reaction with trichophytin with sufficient regularity for the test to be useful for confirming a clinical diagnosis of this condition, and that patients infected by an "animal' species of ringworm fungi, such as *Microsporum canis*, which, as already indicated (p 13), tends to give an inflammatory reaction, most often give a strong response to trichophytin The trichophytin reaction is not, however, specific, infection by one species will usually induce a reaction to trichophytin prepared from another species of the same or a different genus and it is customary for trichophytin to be a blend of several different dermatophytes Also, individuals giving a strong trichophytin response will often give a positive, if weaker, reaction with an extract of another fungus such as *Candida albicans*

Many people give a positive skin reaction to "oidiomycin" prepared from *C albicans* and in view of the widespread occurrence of this fungus in the respiratory and digestive tracts of normal individuals (*see* p 32) this is not perhaps surprising In addition to a positive skin reaction, agglutinin, precipitin, and complement-fixation reactions may also be demonstrated both in those suffering from moniliasis and apparently healthy individuals In general, for moniliasis as for dermatomycoses serological tests have only a confirmatory value in diagnosis Conant *et al* (1945) do, however, recommend that the skin

test should always be made in cases of moniliasis, as potassium iodide therapy, one of the standard treatments for that disease is dangerous to strongly sensitized persons

C E Smith *et al* (1950) found that the combination of precipitin and complement fixation tests can establish the causes of 60 to 99 per cent of infections by *Coccidioides immitis* in man They also found that, as in experimental histoplasmosis in rabbits to which reference has already been made (p 68), precipitin tests are most useful in the early, and complement-fixation tests in the later, stages of the disease Complement fixation attains its maximum titre in disseminated infections and a fall in precipitin titre and a rise in complement-fixation titre may be a useful indication of the dissemination of an infection. Coccidioidomycosis also confers a skin sensitivity This may disappear in the final stages of a fatal infection but it persists for many years in those who recover A positive skin reaction to coccidioidin is not, therefore, usually indicative of active infection but the extensive cutaneous testing undertaken during recent years is a good example of the important information on the geographical distribution and incidence of a mycosis given by mass serological testing In South Arizona where coccidioidomycosis is endemic, more than 97 per cent of Indian children may react to coccidioidin and during the Second World War the development of coccidioidin sensitivity in troops after being stationed for training in the desert region of the United States was frequently observed Recently, Mackinnon and his collaborators (1950) have used the skin test to investigate the distribution of coccidioidomycosis in South America Of 62 individuals from the hot arid zones of the Chaco and other provinces 8 reacted to coccidioidin Coccidioidin-sensitive individuals were also detected in adjacent regions and confirmed the authors in their view that coccidioidomy-

[illegible]

series of 433 from Buenos Aires It is suggested that these low values are due to cross-reaction with *Histoplasma capsulatum* In Buenos Aires 15 per cent of the population is said to be histoplasmin positive and when in Uruguay 360 adults were tested with both antigens, 38 reactors to histoplasmin were detected and only one to coccidioidin, the individual who gave the strongest reaction to histoplasmin No positive reactors to either antigen were obtained in Santiago, Chile.

Histoplasmin skin-testing affords an example of the use of serological methods to elucidate a condition of uncertain etiology In states of the Mississippi basin, from 27 to 83 per cent of young persons were found to have pulmonary calcifications such as are normally associated with tuberculosis Only approximately one-half of those showing such abnormalities gave a positive reaction on testing with tuberculin. Tuberculin sensitivity is considered to be a stable condition and a reliable indication of an attack of tuberculosis Tubercular infections did not, therefore, appear to explain the calcifications in tuberculin insensitive individuals. An alternative explanation was advanced in 1943 by C E. Smith who suggested that histoplasmosis might be endemic in the area This resulted in large scale cutaneous testing with histoplasmin and Iams (1950) has summarized the results of 27,780 recorded tests Of these, 38 6 per cent were histoplasmin positive, 61 4 per cent histoplasmin negative, and no patient showed any signs of active histoplasmosis When the comparative results of histoplasmin and tuberculin tests were analysed in a series of 2,577 patients exhibiting pulmonary calcifications it was found that 13 per cent were histoplasmin and tuberculin positive while 69 5 per cent were histoplasmin positive but tuberculin negative, and 5 3 per cent tuberculin positive and histoplasmin negative That is, 18·3 per cent gave a positive tuberculin reaction and 82 5 per cent a positive histoplasmin reaction

These results suggest that a relationship may exist between histoplasmin sensitivity and the presence of pulmonary calcifications Emmons, Olsen and Eldridge (1945) showed, as did Salvin (*see* Table VII), that histoplasmin gave a cross-reaction with other fungal antigens and particularly with blastomycin A doubt must, therefore, remain regarding the interpretation of a positive histoplasmin reaction although Howell (1947) has shown that different batches of histoplasmin vary markedly in their clinical titres and that good antigens used at their critical titres give few cross-reactions while Furcolow (1950) failed to detect a cross-reaction with blastomycin in cases of human histoplasmosis. Naturally, a watch is being kept for mild or subclinical manifestations of histoplasmosis in areas where the incidence of unexplained pulmonary calcifications is high Emmons, Olsen, and Eldridge reported the isolation of *H capsulatum* from the hilar lymph node of a child with pulmonary calcifications and recently Christie (1950) has recorded the isolation of *H capsulatum* from two infants and its microscopical identification in the lung of a third, cases which he suggests represent the benign or minimal form of the

disease Furcolow (1950) has recorded spontaneous recovery from histoplasmosis in a child

References

Lewis and Hopper (1948) give a useful account of the results of trichophytin testing and Skinner (1947) summarizes the serology of moniliasis The papers cited in the chapter give references to additional information on the coccidioidin and histoplasmin tests

For details of general serology, *see* Topley and Wilson's *Principles of bacteriology*, ed 3, 1947

particles has the reverse effect on fungus spores, presumably due to an increase in the growth of saprophytic moulds under the more humid conditions, and even very heavy rain storms may achieve only a temporary reduction The spore count is, however, maintained at a very low level during periods when the ground is covered by snow (Fig 5) Another factor is the wind which is an important determinant of "spore storms" One of the most impressive and well-documented of these storms occurred on the 6th–7th October, 1937, throughout the eastern U S A when, according to Durham (1938), the weather record showed that air masses from the north-west at an altitude of 2,000–6 000 feet moved rapidly east and south-east traversing Minnesota to the Atlantic seaboard in about fifty-five hours During this time thousands of tons of mould spores were transported several hundred miles At nearly every station east of the Mississippi river the catch of *Alternaria* spores (up to 2,027 per 1 8 cm^2 per 24 hr) was the highest of the year At seventeen of the stations the average increase in spore count was a hundred times that of the daily mean for October Mass movements of cereal rust spores regularly occur in parts of North America and India where they are responsible for initiating outbreaks of plant disease In addition to such major storms, local atmospheric disturbances frequently result in what may be termed "showers" On the afternoon of 5th June, 1951, at Exeter, for example, during a period in which the temperature remained constant at 22°C the total number of *Cladosporium* colonies developing on groups of five malt agar plates exposed for five minutes at 12 noon, 2, and 4 p m were 6, 47, and 3, respectively

The spores present in the air are widely distributed for in addition to the usual terrestrial records there are instances of fungus spores having been trapped from aeroplanes, up to 70,000 feet and over polar regions, and from the bows of a liner in mid-Atlantic In such localities the catch is lower than that from outdoor air at low altitudes on land where the spore content is higher than in mountain air or in still indoor air The spore content of indoor air may, however, attain exceptionally high values in cow sheds and hay lofts or other dusty situations

The list of identified species of fungi recorded from air is long and the list of unidentified fungi even longer In North America much attention has been paid to the incidence of *Alternaria* spores This is probably partly because of the ease with which *Alternaria* spores can be recognized on gravity slides but the evidence does suggest that the spores of this genus, which often predominate in that region, are particularly

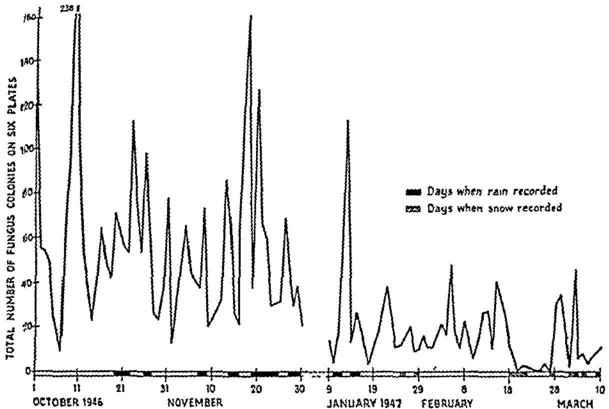


FIG 5 SPORE CONTENT OF THE ATMOSPHERE

Daily fluctuation in the spore content of the air at Sutton, Surrey, during October–November, 1946, and January–February 1947

important as allergens *Alternaria* spores regularly occur in the air of this country (Hyde and Williams, 1946, 1949) but in much smaller numbers, *Cladosporium* spores being the most numerous In Cardiff during 1948 *Cladosporium* colonies accounted for half those developing on air plates, similar results have been obtained in other localities in the British Isles and according to a recent paper by Blackaller (1950) *Cladosporium* is also the predominating airborne fungus in Mexico In addition to species of *Penicillium* and *Aspergillus*, *Pullularia* is frequent and species of *Phoma*, *Botrytis*, *Trichothecium*, etc occur in varying numbers Aerobic actinomycetes and diverse yeasts and bacteria accompany the mycelial forms on plates but phycomycetes such as species of *Mucor* and *Rhizopus* are less commonly recorded than their frepuency in soil and other situations might suggest.

Fungus Spores as Allergens

> *By inhaling the* spores of the Penicillium, in an involuntary experiment, a severe attack of hoarseness, going on to complete aphonia was brought on. This lasted for a couple of days, and ended in a sharpish attack of bronchial catarrh, which almost unfitted me for duty for a day or two. The sensations were so unpleasant that I have never cared to reproduce them

So wrote Blackley in 1873 of an experience which suggested that fungus spores could give rise to an allergic response in man It is clear from the data summarized in the preceding section that fungus spores are always present in the air, sometimes in very large numbers What is the evidence that such airborne spores affect man?

The connexion between airborne pollen and hay-fever which was firmly established by Blackley, if not unanimously accepted at the time, was first suggested by Elliotson as long ago as 1831 Blackley's footnote on his experiences with moulds passed unnoticed and it is only within the last twenty-five years that the importance of mould allergy has become generally recognized

It was in 1924 that Van Leeuwen noted in the island of Zuid-Beveland that 0 5 to 1 0 per cent of the inhabitants of some villages were asthmatic, an incidence greatly in excess of that prevailing in other parts of Holland He failed to associate this condition with any meteorological peculiarity and attributed the cause to "miasmata" or "climate allergens" Further, he showed that sufferers were relieved by air freed from "miasmatic substances" by filtration through cotton wool or by passage through glycerin and he suggested that these airborne

substances were mainly of fungus origin. Also in 1924, Cadham in Canada recorded three cases of agricultural workers who developed severe asthma on exposure to dust from rusted grain and infected straw. He considered the stem rust (*Puccinia graminis*) spores to be the causal agent and he was able to induce a paroxysm by the inhalation of a few rust spores. According to Feinberg (1948), it is possible that the rust spores were contaminated with spores of *Alternaria* or *Cladosporium* because most rust spores are ineffective as allergens and are obtained in pure culture with difficulty. Whether this was so or not, Cadham was able to afford some relief with a vaccine prepared from the rust (Cadham, 1924). In 1930, in the U.S.A., Bernton described a case of asthma associated with *Aspergillus fumigatus* and Hopkins, Benham, and Kesten in the same year described a similar case caused by *Alternaria* spores derived from vegetables stored in the basement of the patient's house. Since then many more instances have been recorded and Durham (1938) states that after the storm of *Alternaria* spores to which reference has already been made "a brief inquiry has disclosed the correlation of clinical symptoms with the advent of the storm in some of the places affected."

The most frequent symptoms of mould allergy are respiratory and usually take the form of bronchial asthma or rhinitis. Conjunctivitis is another common manifestation of mould allergy and in 1939 Feinberg (see Feinberg, 1948) reported fourteen cases of seasonal aggravation of dermatitis in mould-sensitive patients in which there was a positive correlation of fungus spore counts with the intensity of clinical symptoms. Respiratory symptoms usually occur in "summer" and may prevail from April to November in contrast to the more sharply defined attacks experienced by pollen-sensitive subjects. Sometimes all-the-year-round symptoms occur or the symptoms may be confined to the spring and autumn and these patterns may possibly be attributed to sensitivity to fungi such as *Aspergillus* and *Penicillium* which occur in the air at all times of the year (or to co-sensitization to house dust) and to fungi showing seasonal optima, respectively.

A mould-sensitive subject gives a positive skin reaction to one or more fungi or fungus extracts (specific or even generic specificity is rare) and cutaneous testing is the routine method for diagnosis of mould sensitivity. Feinberg states that the scratch test with a 1 : 1,000 extract of the mould (1 gm of dry culture per 1,000 ml normal saline solution) resulting in an immediate whealing type of reaction, is more conclusive than the intradermal test.

Treatment is by desensitization by the administration of increasing doses of fungus extract given once or twice a week. The first injection is 0 5 ml of a 1 : 10,000 dilution and the maximum about 1 ml of a [illegible] dilution The extract used depends on the particular case It [illegible] more usually it is prepared from [illegible] Anti- [illegible] mptoms but the most gener[illegible]

Interest in mould allergy has been greatest [illegible] es where many papers have been published on the subject and whilst the fact that mould allergy is of widespread occurrence and of clinical significance appears to be beyond reasonable doubt much of the data lacks precision This is due in part to the variation in the diagnostic procedures and in part to the difficulty of obtaining proof of the specific allergens involved. In a summary given by Feinberg (1948, p. 275, Table 19) of the results of eleven investigations in the United States involving tests on more than five thousand individuals suffering or suspected to be suffering from allergic disorders, the percentage giving a positive reaction to "moulds" varied from less than 1 to 85 per cent and the reaction of one group diagnosed as suffering from "mould allergy" (75 per cent) was probably not significantly different from that of other groups of "respiratory" and "general allergy" cases which gave 69 and 85 per cent positive mould reactions, respectively Similar results have been obtained in other countries Van Leeuwen in Holland found that half the asthmatics he tested gave a positive reaction to fungi This compares with 15 per cent positive in Germany by Hansen in 1928 and 16 per cent in the same country by Fraenkel in 1938 who obtained 53 per cent of mould reactors in a series in England

In a series of 638 allergic patients examined by Feinberg himself, 261 (40 9 per cent) gave a positive reaction to fungi and because a number of patients had failed to respond to therapy elsewhere he considers that this figure should be cut to about a half and that on a conservative estimate 20 per cent of allergic persons are sensitive to fungi Feinberg also states that 80–90 per cent of his mould-sensitive patients obtain relief from desensitization therapy with mould extracts (and cites claims of 80 and 75 per cent of successes by two other workers) which clearly supports the claim that fungus spores function as allergens.

The fungus spores present in air are normally derived from the saprophytic or parasitic growth of fungi on plants As Hyde and Williams (1946) and others have pointed out, the straw of wheat and other

cereals would appear to be an ideal medium for the growth of *Alternaria* and the predominance of airborne *Alternaria* spores in the states in or adjacent to the wheat belt of central and eastern U.S.A. is, therefore, not surprising while the dominance in other parts of the world of *Cladosporium herbarum*, an ubiquitous and unspecialized saprophyte, is likewise to be expected. In addition to allergic disorders caused by spores of such widespread fungi there are a number of instances of allergy to fungi of more localized distribution.

Cobe, in 1932, described a case of asthma due to the tomato leaf-mould fungus (*Cladosporium fulvum*), the spores of which occur in high concentrations in glasshouses containing affected plants, and similar cases are cited by Brown (1936) and by Morrow and Lowe (1943). The powdery mildew of oak (*Microsphaera alni*) has been associated with allergic disease in California and, according to Feinberg (1948), Sir John Floyer in his *Treatise on Asthma*, 1726, said: 'There is a Remarkable Instance in Bonetus of an Asthmatic, who fell into a violent Fit by going into a wine-cellar where Must was fermenting . . .' which is perhaps the first record of mould allergy. Towey, Sweany, and Huron (1932) reported a series of cases of asthma in Michigan caused by the inhalation of a fungus (identified as *Coniosporium* [*Cryptostroma*] *corticole*) which grew on dead maple trees. It was suggested that in "maple-bark disease" the cough, fever, night sweats, and loss of weight were in part caused by a local toxic effect and in part a delayed effect resembling protein sensitivity. Also, during the war the "*mal des cannes de Provence*" which had been recognized for more than fifty years as an occupational disease of those who handled reeds for thatching became prevalent in the paper mills of the Midi region of France where the reed *Arundo donax* was being used for its fibre. This disease is characterized by lassitude, stiffness, low fever, and slight scrotal swelling. Later a pruriginous erythema spreads upwards from the inner surface of the thighs and becomes particularly severe in the armpits. A cough develops. Work is no longer possible. The patient is obliged to rest. His condition at once improves and recovery is complete in ten to fifteen days. On re-exposure to the reeds there is recurrence of the symptoms. Duché (1944) who investigated this condition attributed the complaint to the response of a sensitized individual to *Coniosporium arundis* [*Papularia sphaerosperma*] which occurs on the lower leaves of reeds of more than one year old. Further, he suggested that the active principle was a brownish black viscid fluid which can be extracted from the fungus.

Moulds and Dust Allergy

During recent years increasing interest has been paid to the industrial hazard of dust. Silicosis in miners is caused by the mechanical action of the mineral particles constituting the dust The cause of bagassosis, an occupational disease of those handling bagasse, the crushed sugar-cane used to make insulating building board and other products after extraction of sugar, is more obscure but this disorder, too, possibly results from some non-allergic effect Other dusts, and particularly house dust, may, however, at least sometimes owe their ability to induce asthma and other disorders to the allergic action of fungus spores

The first published record of dust allergy was that by Cooke in 1922 who described the case of an asthmatic who gave a strong skin reaction and mild asthma to an extract of dust from his room but not to other allergens Cooke was unable to establish the identity of the active fraction in the dust but he found that 109 (or 33 per cent) of a series of 327 asthma patients gave a positive reaction to "dust" (Cooke, 1922) In 1931 Flood tested 55 patients suffering from chronic asthma and obtained 8 positive reactors to fungi isolated from house dust In one patient he precipitated an attack of asthma by spraying the nose and throat with a culture filtrate of *Mucor plumbeus*, the mould to which the patient had reacted Brown (1936), who briefly reviewed the topic of house dust as an allergen cited further examples and inclined to the view that moulds were the cause of dust allergy

The evidence obtained by Rimington and his co-workers in England (*see* Stillwell, Rimington, and Maunsell, 1947) and by Reymann and Schwartz (1947) in Denmark does not, however, support this view Some of the tests made by these investigators are summarized in Table VIII from which it can be seen that 37 of the 38 patients sensitive to moulds were also sensitive to dust but that of the 101 sensitive to dust only 37 were sensitive to moulds Only one patient was sensitive to moulds alone Stillwell *et al* (1947) prepared a "crude dust-antigen' by the extraction of a ton of dust and they found this antigen at 10^{-6} to give an intradermal reaction with 6 per cent of normal persons and with 70 per cent of those with allergic symptoms Further it was shown that subjects with a threshold value of 10^{-7} with the dust allergen showed allergic symptoms *in a dusty atmosphere and treatment by desensitization* with this antigen gave some relief to 16 of 18 patients Additional evidence of the distinctness of the dust and mould antigens is Feinberg's observation that mould allergy is most frequent in children

These facts rather suggest that dust and mould allergy are not very closely related phenomena The relationship may, however, at times

TABLE VIII

SENSITIVITY OF ASTHMATIC SUBJECTS TO DUST AND MOULDS

No of Patients	Dust	Moulds	Polysaccharide from *Penicill um* spp	
22	13 + 9 −	5 + 1 +		Reymann and Schwartz 1947
62	45 + 17 −	15 + 17 −		Stillwell, Rimington, and Maunsell, 1947
36	36 +	12 +		
5 2 5	5 − 2 + 5 +	5 − 2 − 5 +	5 − 2 + 5 +	
132	101 + 31 −	37 + 1 +		Totals

be closer as in the so-called 'farmers' or threshers' lung" which has been noted in country districts of England (Fawcett, 1938) and Scandinavia (Tornell, 1946) This respiratory condition, which

References

The reviews by Brown (1936) and Morrow and Lowe (1943) and the chapter "Allergy to Fungi" in Feinberg (1948) all have long bibliographies. Reference should be made to these publications for the references to work not specifically cited in this chapter and for other details

CHAPTER VIII

POISONOUS FUNGI

A Toxological Problem

THAT some fungi are edible while others are poisonous has been known since earliest times Classical writings contain numerous references to fungi and it is characteristic of human nature that their harmful effects were noted before their use as food was recorded. It is among the larger fungi, particularly the Basidiomycetes (mushrooms and toadstools), that most of the edible species are found A few are Ascomycetes The number of poisonous species is small compared with those which are innocuous Nevertheless, serious illness and even death from eating badly chosen specimens is of frequent occurrence, particularly in countries where the population is less conservative in its taste for fungi than in Britain, so that in parts of western Europe special legislation regulating the sale of fresh and dried fungi is in operation

There is no general rule for the recognition of poisonous fungi except the correct identification of those which past experience has shown to be toxic. Poisonous species are frequently closely related to those which are edible Some genera composed almost entirely of species highly esteemed as food include one or more poisonous forms, e g the genus *Psalliota* includes the poisonous *P xanthoderma*, which closely resembles common mushrooms such as *P arvensis* (the horse mushroom) and *P campestris* (the field mushroom) On the other hand, most species of a genus may be poisonous while a few are edible The genus *Amanita*, for example, includes the highly poisonous *A phalloides*, the poisonous but less dangerous *A muscaria*, and the edible *A. rubescens*

True poisoning should not be confused with inconvenience caused by eating indigestible or old specimens or with individual idiosyncrasy A poisonous fungus may be defined as one which when eaten fresh and in moderate amounts by normal individuals regularly causes definite symptoms of poisoning Cooking or drying alters the poisonous properties of some species (*see Gyromitra esculenta*, p 90) and fungi which are normally edible may become harmful with age due to the chemical changes attending decomposition.

In the present chapter the range of fungus poisoning is illustrated by

a brief consideration of the commoner poisonous basidiomycetes and ascomycetes encountered in this country and of ergotism induced by the inadvertent ingestion of the sclerotia of *Claviceps purpurea*.

Larger Basidiomycetes

More than 90 per cent of the recorded deaths from fungus poisoning are caused by *Amanita phalloides* Poisoning by this species is always serious and frequently fatal, deaths following poisoning being placed at from 50 to 90 per cent. *A phalloides* is common in the woods of this country and Western Europe in late summer and early autumn It also occurs in North Africa and Japan but appears to be rare in North America. The cap of a well-developed sporophore is fleshy and when fully expanded, about 3½ in in diameter It is borne on a stem approximately 4½ in long, varies in colour from olive or [illegible]
a darker centre to bro[illegible]
white, and there ar[illegible]
The gills are crowd[illegible]
with green, bears a well marked ring and has at the base a large white [illegible], which is white or tinged or yellowish papery volva. The mature spores are white (*See* Fig 6)

The characteristic features of *A phalloides* poisoning are well marked For a variable time after ingestion, usually 10–12 hours but sometimes as long as 14–20, no inconvenience is felt. This period of incubation is followed by the sudden onset of severe abdominal pains vomiting, diarrhoea, and great thirst. Painful cramp in the calves, coldness of the extremities, and cyanosis are also characteristic and the clinical picture resembles that of cholera. After this first phase nervous symptoms develop but delirium is rare On about the second day the condition of the patient shows an apparent improvement but the various symptoms usually return two or three days later with increased severity and it becomes evident that the liver is seriously affected The nervous system next becomes paralysed, the patient may lapse into a coma, and death ensues There are many variations of this syndrome one type of symptom commonly predominating The gastro-intestinal symptoms are usually the most important but in some cases complete immobility and prostration predominates, in others there is prolonged coma, delirium, or convulsions, while the heart, liver, and kidneys are all affected in varying degrees. Post-mortem examination shows a lack of rigidity and the blood, which is cherry-red in colour or black and viscous, fails to coagulate The pupils are dilated. Fatty degeneration of the liver and kidneys is common

Much work has been done on the chemical nature of the toxic principle of *A. phalloides* since Boudier in 1846 considered the poison to be an alkaloid. The foundation of present knowledge was laid by Kobert in 1891 who isolated a complex toxic substance (a glucoside) which he named "phallin" and which Ford showed to consist of at least two fractions, amanita-haemolysin and amanita-toxin. The first,

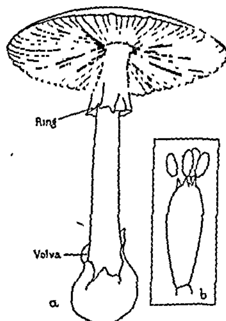


Fig. 6 *Amanita phalloides* Sporophore
a × ⅔ b, Diagram of a basidium and four exogenously developed basidiospores. × 500

a haemolytic poison, is thermo-labile and destroyed by digestive juices, the second is more stable and is not inactivated by the digestive juices or boiling water although it is decomposed by boiling acids More recently, in 1937, Lynen and Wieland isolated three poisons. One of these, which they named "phalloidin," is a hexapeptide and acts more quickly than the other two. In animals it causes lesions of the liver and digestive tract similar to those of *A. phalloides* poisoning in man

Although one mouthful may prove fatal, recovery from *A. phalloides* poisoning does occur. The recovery is, however, always slow and the outcome of the various treatments which have been

recommended uncertain The Pasteur Institute has developed an anti-phalloidian serum This is injected either intravenously or hypodermically in amounts of 40 ml or more Good results are claimed if the injections are given soon enough. Since, however, it is not usually known that *A phalloides* has been eaten until the symptoms of poisoning appear, and since the serum is not always easily procurable, this treatment is of more theoretical than practical interest The observation that rabbits and sheep eat small amounts of *A phalloides* without ill effects has led to the patient being given the fresh brains of seven rabbits and the stomachs of three chopped up together, the explanation advanced being that the poisoning is due to two toxins, one, which acts rapidly on the liver kidneys and intestines, being neutralized by the stomach of the rabbit, the other acting more slowly on the nervous system being neutralized by the rabbit brains Though repulsive to the patient, cures have been effected by this means in France

Glucose injected intravenously, is used in Germany against *A phalloides* poisoning and this treatment has met with success both in clinical cases and in experiments with animals The reason for its success is not fully understood for though hypoglycaemia is caused by the poisoning this does not seem to be an essential feature Another little understood treatment for which successes have been claimed is that of giving common salt, either as intravenous injections or orally

Two other fungi which are closely allied to *A phalloides* and which cause the same type of poisoning are *A verna* and *A virosa* In all three the effect of the poison is to cause degeneration of the cells of the internal organs, particularly the liver and kidneys and death frequently follows The syndrome of *Lepiota helveola* poisoning resembles that caused by *A phalloides* but *Volvaria gloiocephala* and *A citrina*, both at one time thought to cause poisoning of this type, are now known to be non-toxic

Amanita muscaria and *A pantherina* are both responsible for poisoning of a very different type They act predominantly on the nervous system and gastro-intestinal symptoms, though present, are usually of secondary importance Of the two, *A pantherina* causes the more serious poisoning and a death rate of 10 to 20 per cent is reported by some authorities *A muscaria*, although the popular prototype of a poisonous fungus, is not fatal if the subject is healthy

A muscaria, the fly agaric (so named because it is lethal to flies), is well known on account of its bright scarlet or orange-red cap on the

surface of which are scattered whitish, wart-like masses The gills and stem are white There is a well-developed ring but the volva is represented by only a concentric series of white or yellowish squamules above the bulbous base of the stem The colour of the cap of *A panthenna* varies from yellowish-brown to brownish-grey The stem is white with a bulbous base which shows the remains of a volva and the obliquely attached ring does not persist

In contrast to *A phalloides* poisoning, the onset of symptoms occurs without delay, usually *within 3 hours of the meal Vomiting and* diarrhoea are accompanied by delirium and hallucination which is both visual and auditory These continue for about four hours and are followed by a deep stupor and sleep from which the patient wakes feeling much better and with no recollection of his illness Sometimes the digestive symptoms are more pronounced and in such cases the poisonous matter is quickly eliminated from the system and recovery is rapid More rarely there is no digestive upset and then the delirium is more serious

The effect of the fly agaric on the nervous system has long been known In 1560, the herbalist Bauhin called this fungus "the fungus of folly" and it is greatly esteemed by the Koryak tribes of north-east Siberia where it is eaten to induce excitement and intoxication The first poisonous principle to be isolated from *A muscaria* was muscarine (myceto-muscarine) which also occurs in *A panthenna* It has since been prepared experimentally by the oxidation of choline which is also present in the fungus in fairly large amounts and which is probably *responsible for the digestive troubles usually accompanying A muscaria* poisoning Although muscarine induces characteristic symptoms, particularly excessive excretion by the sudorial and salivary glands, diarrhoea, and slowing of the heart, they are not the chief [illegible] ated with *A m scaria* poisoning These are generally [illegible] induce [illegible] *uscaria* [illegible] French workers as the "pantherine syndrome" to distinguish it from the "sudorian syndrome" induced by muscarine

In addition to *A muscaria* and *A panthenna*, muscaridine appears to be the active principle in *Clitocybe dealbata C rivulosa*, and in species of *Inocybe*, e g *I patouillardu* which may be fatal, while muscarine is present in agarics such as *Stropharia semiglobata*, exceptionally in *C dealbata*, and in the tube-bearing fungus, *Boletus calopus*

The treatment for poisoning by *A muscaria* and other fungi having a like effect is broadly speaking that for violent indigestion. Vomiting is spontaneous and should not be induced but purgatives may be administered to expel any remaining fungus Potassium bromide and chloride are given against the nervous symptoms and the injection of ether or other stimulant combats cardiac depression. The North American form of *A muscaria* appears to contain larger amounts of muscarine than do European specimens and against this atropine is effective but this drug should not be administered in cases of poisoning by the fly agaric in Britain.

Clinical symptoms have frequently been used as a basis for the classification of poisonous fungi and although such classifications are useful they are very artificial Symptoms which usually predominate in poisoning by a given fungus are often modified by idiosyncrasy of the patient so that they are only a guide to the species involved Microscopic examination of the vomit and stools for spores or fragments of fungus may provide more reliable diagnostic evidence

The clinical classification devised by Roch has been used by Dujarric and Heim (1938) who recognize three categories of poisonous agarics—

1 Fungi which after a long incubation-period cause the degeneration of the cells of the internal organs, e g *Amanita phalloides*, *A verna*, *A. virosa*

2 Fungi which affect the nervous system directly, e g *A muscaria*, *A. pantherina*

3 Fungi causing gastro-enteritis, e g *Entoloma lividum* which is sometimes fatal

There are many other basidiomycetes which have a mildly poisonous action on man and may be considered as indigestible and therefore best avoided With most of these the symptoms are confined to nausea, vomiting, colic (which may be painful), diarrhoea and sometimes nervous or other troubles but these last are usually of secondary importance *Tricholoma pardinum* causes violent and painful enteritis which may persist for as long as six days, and several species of *Russula* and *Lactarius* have a decidedly irritating effect on the digestive tract. Some European species of *Boletus* are able to cause gastro-intestinal upsets and the polypore *Fomes officinalis* has long been known for its purgative action. Reports of poisoning by other basidiomycetes are not uncommon but the evidence as to the identity of the species eaten is often unreliable and conflicting

Of a rather different character are the troubles associated with *Coprinus atramentarius* one of the common inkcaps Although this fungus can be eaten with impunity by some people with others if taken with alcohol it is said to have produced cardio-vascular erethism, congestion and cyanosis of the face and scalp The symptoms die away after some hours If however, more alcohol is then drunk they may re-appear It has been suggested that the poisonous substance is soluble in alcohol but this does not explain the sporadic nature of the attacks There is no satisfactory explanation of the facts

Larger Ascomycetes

The most esteemed esculents among the larger ascomycetes are truffles (the underground fructifications of species of *Tuber*) and *Morchella esculenta* the morel Rather similar to the latter is *Gyromitra esculenta* This fungus which causes poisonings which may be fatal has a deeply convoluted cap fawn-coloured when young and dark chestnut in older specimens. The surface of the cap turns in to meet the whitish stem which is flattened somewhat and longitudinally grooved If cooked or dried the poisonous properties of *G esculenta* are lost though poisonings do occur when the cooking water is served with the dish. For this reason the number of cases reported in France where the fungus is eaten cooked or dried is small Poisonings are more frequent in Germany where the fungus is eaten raw or after little cooking and cases resulting in death have been reported from North America There is always a long incubation period after the ingestion of the fungus varying from eight hours to two days and this is followed by vomiting diarrhoea nervous troubles and icterа and alternating periods of deep sleep and convulsions the liver and spleen are swollen and the pulse slow and irregular A coma may precede and continue up to the time of death

It was first thought that poisoning is due to a haemolytic toxin helvellic acid which was isolated by German workers before the end of the last century The acid is destroyed at temperatures of about 60°C and by drying and this would explain why cooked and dried specimens lose their toxicity On the other hand it is known that the poison does at times occur in the cooking water and recent work indicates that the problem is a complex one It seems likely that a second thermostable poison is present and that this alone is able to cause death Some workers even state that no haemolytic symptoms occur in man Personal idiosyncrasies here too would account for

some of the contradictory evidence (some people are able to eat *G esculenta* raw and children are more liable to poisoning than are adults) but not for all. One suggestion is that poisonings are anaphylactic in nature since poisoning often occurs when a second meal of the fungus is taken shortly after the first.

Ergotism

Spores of the ergot fungus (*Claviceps purpurea*, an ascomycete) infect flowers of cereals, particularly rye, and of grasses As a result, the ovary is destroyed and the "seed" replaced by a sclerotium (a compacted mass of hyphae) which projects from the ear as a small, dark, horn-like structure (*see* Fig 7)

These sclerotia, when derived from rye, may be 1 5 to 3 5 (or more) cm in length and constitute the ergot of commerce They contain several alkaloids able to stimulate plain muscle to contract and it is to this property that ergot owes its employment in gynaecology If inadvertently eaten by cattle or by man characteristic signs of poisoning result and death may follow Losses in stock are usually due to infected grass while poisoning in man normally results from the use of ergot-contaminated rye flour



Fig. 7 Ear of Rye S[illegible] Sclerotia of Clavicep[illegible] ×

Ergot poisoning in man is now rather rare One of the more recent outbreaks was that which occurred in Manchester in the autumn of 1928 when there were 200 cases of ergotism among Jewish grants from central Europe whc 1928, Morgan, 1929) Tl had been grown in south cent of ergot by hand-p of a rye-bread eater in bread-making was

ergot The rye had been grown in Lancashire in 1922, another wet year (Dilling and Kelley, 1928) Formerly ergotism was more prevalent and more severe and during the Middle Ages it was known in Europe as St Anthony's Fire or the Fire of St. Martial.

Two types of ergotism have been distinguished, the *gangrenous* and the *convulsive* The former begins with a general lassitude, vague lumbar pains or pains in a limb, especially the calf, and the intellect is dulled In the course of a few weeks the affected extremity becomes swollen and inflamed and attacked by burning pains heat alternating with icy cold Gradually the affected part is numbed and later becomes gangrenous, mummified, and dry The gangrene spreads upwards and the gangrenous limb may separate spontaneously without the loss of blood

Gangrenous ergotism in Europe occurred west of the Rhine East of that river ergotism was usually of the convulsive type in which convulsive twitching of the hands and feet or of the whole body was accompanied by terrible pains In the intervals between the convulsions there was little discomfort, a ravenous hunger (*there is a record of two patients each consuming three pounds of bread within seven minutes*) and severe insomnia If not fatal, convalescence is protracted (Barger 1931)

In the first of the British cases mentioned above, the symptoms were of the mild nervous type while in the second the Liverpool case, there was gangrene of symmetrical toes It is thought that deficiency in vitamin A is probably a factor which determines convulsive ergotism. This would explain repeated outbreaks of this type in orphanages and prisons where the diet was inadequate and there is a positive correlation between the greater availability of dairy products (which are the chief source of vitamin A) in France than in Germany and the type of ergotism characteristic of the two countries

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The monograph by Dujarric de la Rivière and Heim (1938), with its extensive bibliography, gives a comprehensive account of the poisonous basidiomycetes and larger ascomycetes of Europe This may be supplemented by Ford (1923), Rautavaara (1940) and the useful semi-popular booklet by Ramsbottom (1945) Ergot and ergotism are exhaustively treated by Barger (1931)

EXPLANATION OF THE PLATES

PLATE I

Black piedra (*Piedraia hortai*)

1 Naturally infected hair showing black fungus nodules

2–5 Growth of *P hortai* on Sabouraud's agar after 8 17, and 30 days and 4 months

6 Nodule on hair after treatment with caustic potash solution.

7 Part of a nodule showing development of asci and ascospores.

8–21 Stages in ascus and ascospore development

(Horta (1911) plate 5 Reproduced by permission of the author and the Instituto Oswaldo Cruz)

PLATE II

Trichophyton mentagrophytes (= *T* (*gypseum*) *asteroides* of Sabouraud)

I Primary culture after 40 days on Sabouraud's maltose agar

II–III. Overgrowth of the primary culture by the non-sporing pleomorphic form.

IV and IV[a] Culture of the pleomorphic form on Sabouraud's maltose agar after 13 and 20 days respectively

(Sabouraud (1910) plate 1 Reproduced by permission of Masson et Cie, Paris)

PLATE III

Tinea imbricata (*Trichophyton concentricum*)

1 Tinea imbricata on a Brazilian Indian. (Photograph by Dr A E. de Arêa Leão and Dr M Goto)

2 [illegible]

[illegible]

PLATE IV

Tinea capitis (*Microsporum canis*) Lesion on boy's head (Photograph by Dr Clara M. Warren.)

PLATE V

Microsporum canis

1 Naturally infected hair after treatment with caustic potash to show sheath of irregularly arranged arthrospores Unstained × 500

2 Profuse development of macroconidia on fragment of hair after incubation in a hanging drop for 6 days Unstained lactophenol mount. × 250

PLATE VI

1 Black grain "Madura foot." External appearance of foot. Black grains may be seen in the exudate. A white bristle has been inserted in one of the openings (Carter (1873) plate II (1))

2 *Madurella mycetomi* granule in tissue of foot. ×250 (Preparation by Dr J T Duncan)

3 Granule of *Actinomyces* in lung tissue ×250 (Preparation by Prof Floriano P de Almeida.)

PLATE VII

1 Chromoblastomycosis (*Phialophora* sp) of leg (Photograph by Dr A. E. de Arêa Leão)

2 Cells of *Phialophora* in tissue ×500 (Preparation by Prof Floriano P de Almeida.)

3 Sporotrichosis of arm. *Note* the primary lesion on the hand and the succession of secondary abscesses along the arm. (Photograph by Dr M A F Helm. Reproduced from *Sporotrichosis* 1949 by permission of the Transvaal Chamber of Mines)

PLATE VIII

1 Airborne fungus spores, etc, trapped outside the Presidency Jail, Calcutta, by D D Cunningham on 27th May, 1872 Temp 90 6°, wind, S by E. and S, rain, 0·0 in ×270 (Cunningham (1873), plate V (4))

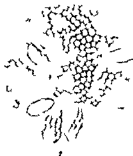
2 Fungus colonies on plate of malt agar exposed to the outdoor air for 5 minutes on 23rd August, 1951, at Exeter, and then incubated for 6 days at 25°C

3 *Cryptococcus neoformans* in human brain tissue Note the wide capsule around each cell of the parasite ×500 (Preparation by Dr J T Duncan.)

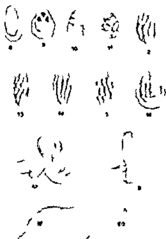
4. *Paracoccidioides brasiliensis* Multiple budding in tissue ×500 (Photograph by Prof J E Mackinnon)

5 *Coccidioides immitis* spherule in tissue ×500 (Preparation by Prof. Floriano P de Almeida.)









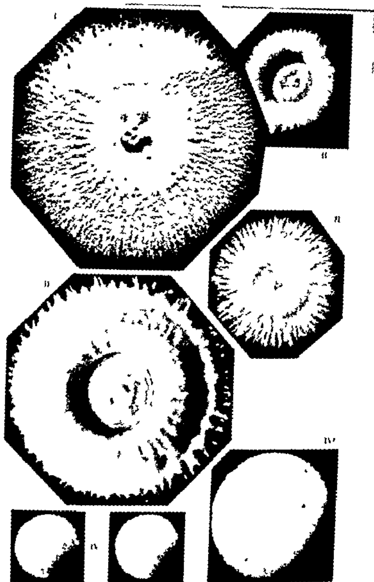


PLATE II

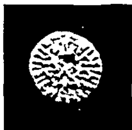
Tr chophyton mentagrophyte



1 T nea mb a a



2 T o en um n sk n scale



3 T omen um n cul ure

I LATE III

TINEA IMBRICATA (*T ichophy n concentricum*)



PLATE IV

TINEA CAPITIS (*Microsporum canis*)



1 Arthrospores (× 500)



2 Macroconidia (210)

PLATE V

Microsporum canis



1 Bla k gra n M dura foot

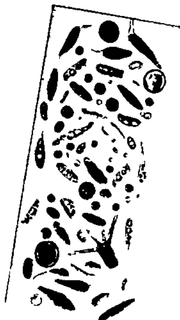


2 Mad re a my et g ain in t ue



3 A n nyces grain in lung sue

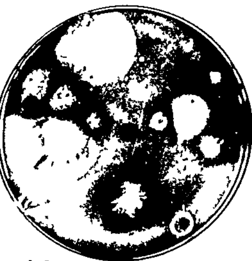
PLATE VI



1 Airborne spores



3 *Cryptococcus neoformans* in brain



2 Colonies from airborne spores



4 *Paracoccidioides brasiliensis*
Multiple budding



5 *Coccidioides immitis* spherule

PLATE VIII

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